The Journal of
Allergy
VOL. 30, NO. 4
JULY—AUGUST, 1959

ORIGINAL ARTICLES

The Effects of a Ragweed Control Program on Ragweed Pollen Counts

Edward M. Cohart, M.D., New Haven, Conn., and Roscoe P. Kandle, M.D., New York, N. Y.

NEW YORK CITY has been engaged in a program of ragweed control for a number of years. Despite the fact that the ragweed control efforts have fallen far short of the goal of complete or almost complete elimination of ragweed within the city, the program was costly. In 1956, when only 35 per cent of the estimated 9,000 acres of ragweed in the five boroughs of New York City were treated, the approximate total cost of the program was $92,000.00. There arises the obvious question: "What are the city's taxpayers getting for this money?"

Programs that have emotional appeal and the support of groups of dedicated persons, such as the hay fever sufferers, are most likely to be badly distorted by hunches or theories in the absence of facts. Prominent allergists, as well as some members of the staff of the Department of Health, have been critical of both the basic premises of the ragweed control program and its operation in New York City. Therefore, the Department of Health decided to attempt an evalaution of the New York City program during the summer of 1956.

The study was designed to test two hypotheses, based on the medically accepted dictum that ragweed hay fever is caused by ragweed pollen. First, the study proposed to determine whether the almost total eradication of ragweed in one borough would result in materially lower pollen concentrations in that borough than in the others not so treated. Second, the study was designed to provide information on whether ragweed hay fever sufferers in the treated borough experienced less frequent or less severe symptoms than hay fever sufferers in the other boroughs. The study of ragweed pollen concentrations is the subject of this article. The relationship of control efforts to symptoms will be discussed in a subsequent report.

From the Department of Public Health, Yale University, and the Department of Health of the City of New York.
Received for publication Dec. 2, 1958.

A third hypothesis, which the study was unsuccessful in testing, was suggested by Dr. Roger P. Wodehouse of the Lederle Laboratories. Laboratory experience had shown that the antigenicity of pollen decreased on exposure to

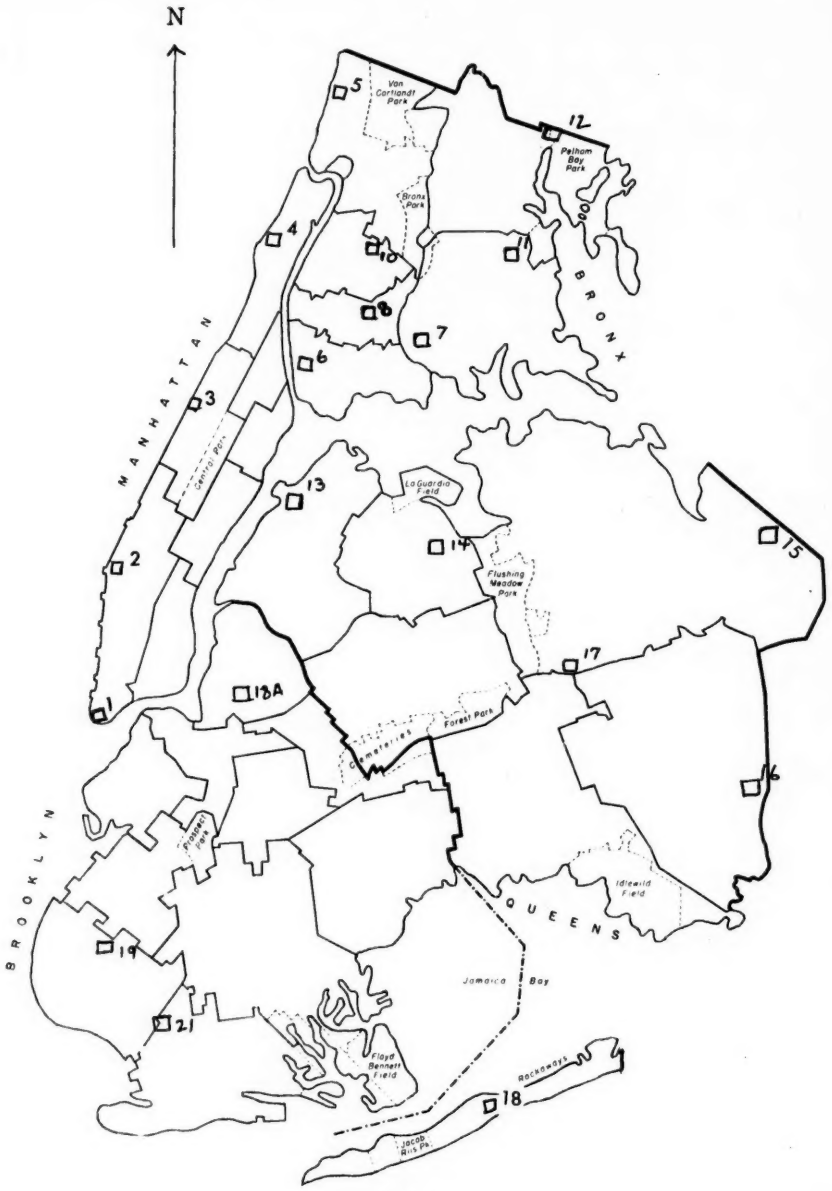


Fig. 1.

atmospheric conditions and with age as measured in days. On the basis of these laboratory findings, the hypothesis was advanced that pollen transported for considerable distances by wind currents and subjected to periods of drying

or wetting loses antigenic potency. If this assumption were correct, a grain of pollen from a neighborhood plant would be a much more potent producer of symptoms than a grain of pollen that had been wind-borne over many miles. Unfortunately, the study was not able to collect and supply Dr. Wodehouse with enough material to decide this question of antigenicity.

METHOD

New York City covers an area of 319 square miles: 41 in the Bronx, 80 in Brooklyn, 22 in Manhattan, 119 in Queens, and 57 in Richmond. Because only one pollen collecting station was established in Richmond, data from this borough were not included in the analysis.

In 1956 the Bronx was estimated to have 2,000 acres of ragweed; Brooklyn, 2,500 acres; Manhattan, 35 acres; and Queens, 2,660 acres. An intensive program of ragweed eradication was undertaken in the Bronx, while "token" programs were carried out in the other boroughs. Treatment, begun on June 20, consisted of spraying with 2, 4-D. The "token" program in Manhattan succeeded in spraying 100 per cent of the very limited ragweed acreage in that borough. In the Bronx, 97 per cent of the ragweed acreage was treated; in Queens, 31 per cent; and in Brooklyn, 6 per cent.

TABLE I. LOCATION OF TWENTY-ONE RAGWEED POLLEN COLLECTING STATIONS IN FOUR BOROUGHS OF NEW YORK CITY IN AUGUST AND SEPTEMBER, 1956

STATION NO.	BOROUGH	LOCATION
1	Manhattan	Old Slip and South St.
2	Manhattan	303 Ninth Ave.
3	Manhattan	150 West 68th St.
4	Manhattan	600 West 168th St.
5	Bronx	6 Peret St.
6	Bronx	349 East 140th St.
7	Bronx	661 Prospect Ave.
8	Bronx	1309 Fulton Ave.
10	Bronx	1826 Arthur Ave.
11	Bronx	2527 Globe Ave.
12	Bronx	4109 White Plains Ave.
13	Queens	12-26 31st Ave.
14	Queens	34-33 Junction Blvd.
15	Queens	44-01 244th St.
16	Queens	254-20 Union Tpke.
17	Queens	90-37 Parsons Blvd.
18	Queens	67-10 Rockaway Beach Blvd.
18A	Brooklyn	88 India St.
19	Brooklyn	186 Grove St.
20	Brooklyn	7918 Third Ave.
21	Brooklyn	1601 Avenue S

A number of pollen collecting stations were established in each borough. Seven stations were established in the Bronx, six in Queens, and four each in Brooklyn and Manhattan. The location of the stations is recorded in Table I and Fig. 1. Slides were collected according to the gravimetric method of Durham.

Arrangements were made for the daily collection of slides from each of the stations, but because of administrative difficulties only 84 per cent of the

daily counts from the total of twenty-one stations were obtained. In Brooklyn, 78 per cent of the slides were collected and read; in Manhattan, 81 per cent; in Queens, 86 per cent; and in the Bronx, 87 per cent. The percentage of the total possible daily pollen counts actually obtained in the stations of the Bronx and Queens was significantly* higher than in Brooklyn and Manhattan. A more detailed record of the percentage of daily pollen counts obtained appears in Table II.

TABLE II. PERCENTAGE OF TOTAL POSSIBLE DAILY POLLEN COUNTS OBTAINED

DATE	BRONX	BROOKLYN	MAN-HATTAN	QUEENS	FOUR BOROUGHS
August					
1-10	81.4	85.0	92.5	85.0	85.2
11-20	90.0	77.5	87.5	88.3	86.7
21-31	93.5	92.5	79.5	95.4	89.6
September					
1-10	81.4	75.0	85.0	78.3	80.0
11-20	88.6	85.0	77.5	86.7	85.2
21-30	88.6	62.5	62.5	78.3	75.7
August and September	87.4	78.3	80.7	85.5	83.8

The incompleteness of pollen count data presented some difficulties in borough comparisons. On those days on which no pollen counts had been made in any of the collecting stations in a borough—and this occurred on only three days in Brooklyn—obviously no comparisons could be made between the pollen counts of that and other boroughs. For other days on which only partial data were available, the missing counts were estimated on the basis of the actual counts on that day in the other collecting stations in the borough and the general relative magnitude of the counts of these other stations to the counts obtained in the station for which the estimate was being made.

These estimates are admittedly crude. In defense of their use, it can be said that (1) necessity dictated some such treatment of the data; (2) estimated counts constituted only 15 per cent of the total counts and therefore did not greatly influence the results obtained; and (3) the general method of using an average of counts from a number of collecting stations in a borough is, at best, no more than a crude index of pollen concentrations in that locality. A different location of collecting stations in the borough might very well have yielded different results. Really valid data for any area of this size can be obtained only by employing many more collecting stations located throughout the area on a random or representative basis. If ragweed hay fever, rather than pollen concentrations themselves, is the ultimate objective of the study, then consideration must be given to concentration of population as one of the variables in choosing a representative sample. The Department of Health was not prepared to undertake an extensive study of this nature but felt that the method used, crude as it is, might still yield revealing information for the purposes of this study.

*Throughout this article an occurrence that could be attributed to chance less than one time in twenty is considered significant.

RESULTS

Data from twenty-one collecting stations in New York City (excluding the borough of Richmond) showed no ragweed pollen during the first week of August. Small amounts of pollen were reported for the first time from one of the stations in the city on August 8. After this date an increasing number of stations began to report pollen, so that the average ragweed pollen counts for the city tended to build up somewhat irregularly until counts of 1 pollen grain per square centimeter on August 14 and 2 grains per square centimeter on August 16 were reached (Table III, and Fig. 2). The first minor peak occurred on August 17, when the average count rose to 6; the count then declined somewhat irregularly to 2 on both August 22 and August 23.

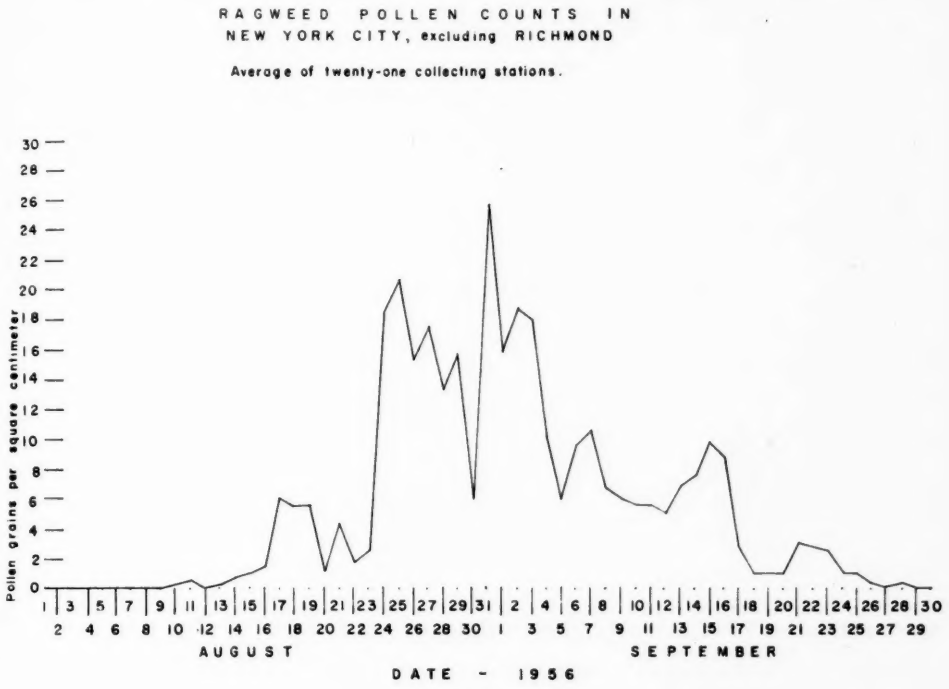


Fig. 2.

The first major peak occurred on August 24 with an abrupt rise of the count to 19. On August 25 the count was 20. It then fell in an irregular, steplike fashion and reached a low of 6 on August 30.

The highest count of the season, 26, occurred on August 31. This was a sharp rise from the day before and was followed by a rather sharp fall to 16 on September 1. The count then remained at about this level on September 2 and 3, showed a steep decline on September 4, and reached 6 grains per square centimeter on September 5.

For the following eleven days, from September 6 to and including September 16, the count varied between 5 and 10. From September 17 on, the

TABLE III. RAGWEED POLLEN COUNTS AND WEATHER CONDITIONS IN NEW YORK CITY, EXCLUDING RICHMOND, IN AUGUST AND SEPTEMBER, 1956

DATE	WIND DIRECTION (1)	WIND VELOCITY (2)	TEMPERATURE (3)	HUMIDITY (4)	PRECIPITATION (5)	NEW YORK CITY, EXCEPT RICHMOND (6)	BRONX (7)	BROOKLYN (8)	MANHATTAN (9)	QUEENS (10)
August										
1	S	7.8	76	76	0	0	0	0	0	0
2	N	11.2	73	60	T*	0	0	0	0	0
3	N	9.1	71	66	0	0	0	0	0	0
4	SW	11.0	72	62	0	0	0	0	0	0
5	E	8.3	67	82	0.13	0	0	0	0	0
6	NE	8.1	68	96	0.70	0	0	0	0	0
7	NE	10.2	73	91	T	0	0	0	0	0
8	W	12.8	78	84	0	0.1	0	0	0.5	0
9	SW	13.5	80	70	0	0.1	0	0	0.5	0
10	W	15.3	80	89	0.05	0.3	0	0.2	0	0.8
11	W	13.5	78	67	0	0.4	0.6	0	0	0.7
12	SW	10.3	80	77	T	0.1	0.3	0	0	0
13	SE	9.5	75	71	0.07	0.3	0.3	0.8	0	0.3
14	W	15.0	77	90	0	0.8	0.9	1.0	1.0	0.3
15	W	9.0	80	64	0	0.9	0.9	1.0	0.5	0.8
16	S	8.0	80	60	0	1.6	1.3	3.5	1.2	0.8
17	S	6.0	81	86	0	6.0	6.0	10.8	4.0	4.2
18	S	12.8	81	74	0.10	5.4	6.9	2.5	5.8	5.3
19	NW	17.7	80	91	T	5.4	8.4	2.5	5.2	3.8
20	NE	7.8	68	56	0.23	1.0	1.7	0.5	0.8	0.5
21	NE	5.1	63	92	0.97	4.3	7.3	1.0	7.5	0.8
22	SE	9.6	66	86	0	1.8	2.4	1.8	0.8	1.7
23	SE	12.0	66	78	0	2.5	4.1	1.2	1.0	2.3
24	NW	15.2	70	81	0	18.6	17.9	18.5	11.8	24.0
25	W	11.3	68	64	0	20.4	14.3	29.5	14.0	25.7
26	SW	10.0	69	69	0	15.3	19.0	†	11.2	13.7
27	SW	8.7	74	76	T	17.6	17.1	11.2	26.5	16.5
28	W	14.4	79	81	0.56	13.3	11.3	12.0	13.8	16.2
29	S	10.7	77	90	0.01	15.4	10.7	17.8	13.2	20.7
30	E	9.2	72	96	T	5.9	6.7	5.2	5.8	5.3
31	SE	11.9	76	97	0.03	25.5	27.7	26.8	13.0	30.3
September										
1	SE	11.8	75	94	0.01	15.7	18.6	20.0	10.5	12.8
2	SE	9.7	76	95	0.06	18.5	23.7	20.0	13.5	14.7
3	N	8.3	75	84	0	17.8	18.1	20.0	14.8	18.0
4	S	6.9	75	82	0	10.1	12.4	4.0	7.2	13.3
5	S	7.2	77	96	0	6.1	9.6	3.2	5.2	4.5
6	S	11.4	75	92	0.46	9.6	9.7	4.5	6.2	15.0
7	NW	14.5	66	81	0.01	10.4	12.9	6.5	7.0	12.3
8	NW	16.5	63	64	0	6.7	7.9	0.8	6.5	9.3
9	NW	18.0	57	69	0	5.9	5.6	0.8	6.8	9.2
10	S	10.0	59	70	0	5.5	4.4	4.8	4.8	7.8
11	S	13.9	62	72	0	5.6	4.9	4.0	7.2	6.3
12	S	11.2	69	78	0	5.0	5.9	4.2	4.8	4.7
13	SE	13.3	70	91	0	6.7	6.1	5.0	6.2	8.8
14	W	16.4	77	88	T	7.5	5.4	7.0	3.8	12.7
15	NE	9.4	64	71	0.43	9.8	11.1	11.5	3.8	11.0
16	NE	10.5	65	97	0.05	8.8	5.4	11.5	4.0	14.0
17	W	11.2	69	100	0.03	2.8	2.9	1.0	2.2	4.2

(Continued on next page.)

TABLE III. CONT'D

DATE	WIND DIRECTIONS (1)	WIND VELOCITY (3)	TEMPERATURE (3)	HUMIDITY (4)	PRECIPITATION (5)	NEW YORK CITY, EXCEPT RICHMOND (6)	BRONX (7)	BROOKLYN (8)	MANHATTAN (9)	QUEENS (10)
18	W	18.4	60	73	0	1.1	1.3	0.2	2.2	0.8
19	SE	16.0	61	66	0	1.0	0.7	1.0	1.2	1.3
20	NW	18.5	55	93	0.20	0.8	0.3	0.8	1.2	1.2
21	SW	11.1	52	79	0	2.9	3.1	2.8	2.8	2.7
22	SW	9.8	59	67	T	2.8	2.3	+	2.8	3.3
23	SW	10.7	71	78	0.09	2.6	3.4	+	2.2	2.0
24	NW	12.1	65	91	0.12	1.0	1.3	0.2	1.5	1.0
25	NW	11.5	61	72	0	1.0	0.4	0.5	0	2.7
26	NE	7.9	56	80	0	0.2	0	0.2	0	0.7
27	NE	11.9	53	69	0.38	0.1	0.1	0	0	0.2
28	NE	11.8	54	80	T	0.3	0	0.2	0.2	0.7
29	N	9.5	63	77	0	0.1	0	0	0	0.5
30	SW	10.9	59	93	0	0.1	0	0	0	0.2

(1) = Prevailing winds during the twenty-four-hour period.
(2) = Average velocity in miles per hour during twenty-four-hour period.
(3) = Daily mean temperature.
(4) = Daily mean humidity.
(5) = Precipitation in twenty-four hours.
(6) = Average ragweed pollen count of twenty-one collecting stations.
(7) = Average ragweed pollen count of seven collecting stations.
(8) = Average ragweed pollen count of four collecting stations.
(9) = Average ragweed pollen count of four collecting stations.
(10) =Average ragweed pollen count of six collecting stations.
*T = Thunderstorm.
†No information available from any Brooklyn collecting station on this day.

count varied between 1 and 3 until September 26, when it reached 0 once again, although an occasional station reported finding small amounts of pollen.

If the period of significant ragweed pollen concentrations is considered to have extended from August 17 through September 16, a period when, with three exceptions, average daily pollen counts for the city were 5 or higher, the prevailing winds blew in from the Atlantic Ocean (that is, from the south or southeast) on fifteen days during the period. On the remaining sixteen days the prevailing winds came from New Jersey, Westchester, Connecticut, and Long Island. On ten days winds were from New Jersey to the west, southwest, and northwest; on five days they came from Westchester and Connecticut to the north and northeast; and on one day the prevailing winds were from Long Island to the east.

The average pollen count on the fifteen days when the prevailing winds blew into New York City from the Atlantic Ocean was 9.3, whereas it was 10.5 on the other sixteen days when the winds reaching New York City came from the direction of neighboring land areas. The difference in means was not statistically significant. On the ten days on which the prevailing winds came from New Jersey on the west, southwest, and northwest the average pollen

count was 12.1, but the difference between this count and the count on the days on which the prevailing winds came from the Atlantic Ocean was again without statistical significance.

Furthermore, the highest average pollen count was obtained on a day when the prevailing winds were from the southeast, the next highest on a day when the prevailing winds were from the west, and south or southeast winds prevailed on four of the ten days with the highest pollen counts.

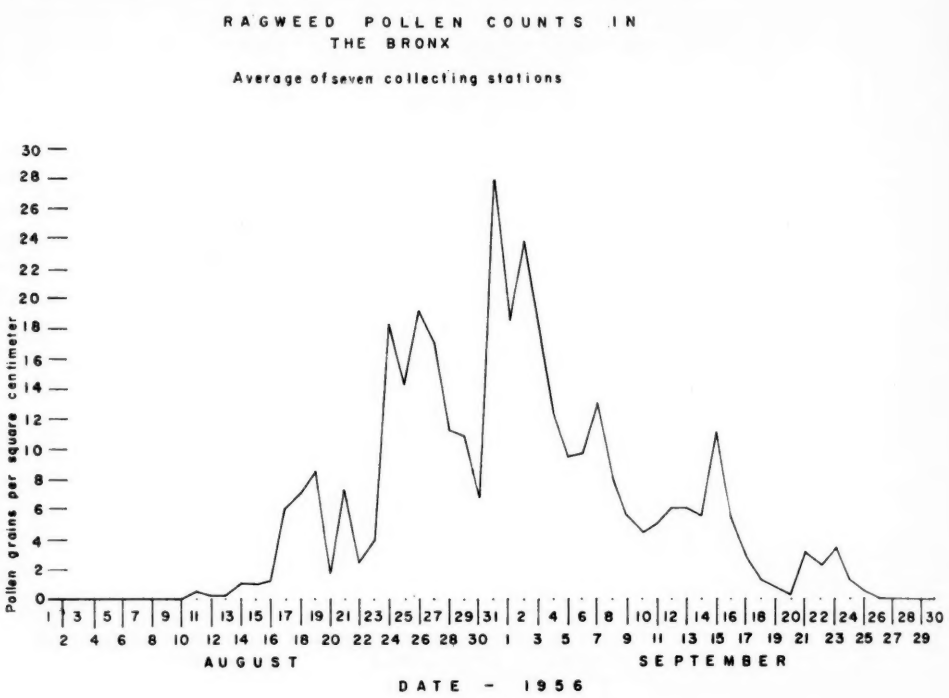


Fig. 3.

Another way of looking at this problem is to relate the prevailing winds to the occurrence of an increase or decrease in pollen count from that of the previous day. Eight of the fifteen days on which winds were southerly or southeasterly showed an increase in pollen count over the previous day, while seven showed a decrease. On the sixteen days when the winds blew into New York City from the surrounding land areas, the counts rose over that of the previous day on seven days, fell on eight days, and showed no change on one day.

Contrary to expectations, there is no evidence from the data accumulated in New York City in the summer of 1956 that winds reaching New York City from neighboring land areas were associated with any higher concentrations of ragweed pollen in the city than winds blowing in from the Atlantic Ocean. Wind velocity, temperature, humidity, and rainfall also could not be shown to have any correlation with the ragweed pollen counts. A continuing study over a number of seasons would be necessary, however, before statements about the absence of such relationships could be made with any assurance.

The average daily pollen counts in four boroughs of New York City are shown in Table III and are presented graphically in Figs. 3, 4, 5, and 6. While there are many differences among the four boroughs in the detailed configurations of the graphs, their general shapes are quite similar.

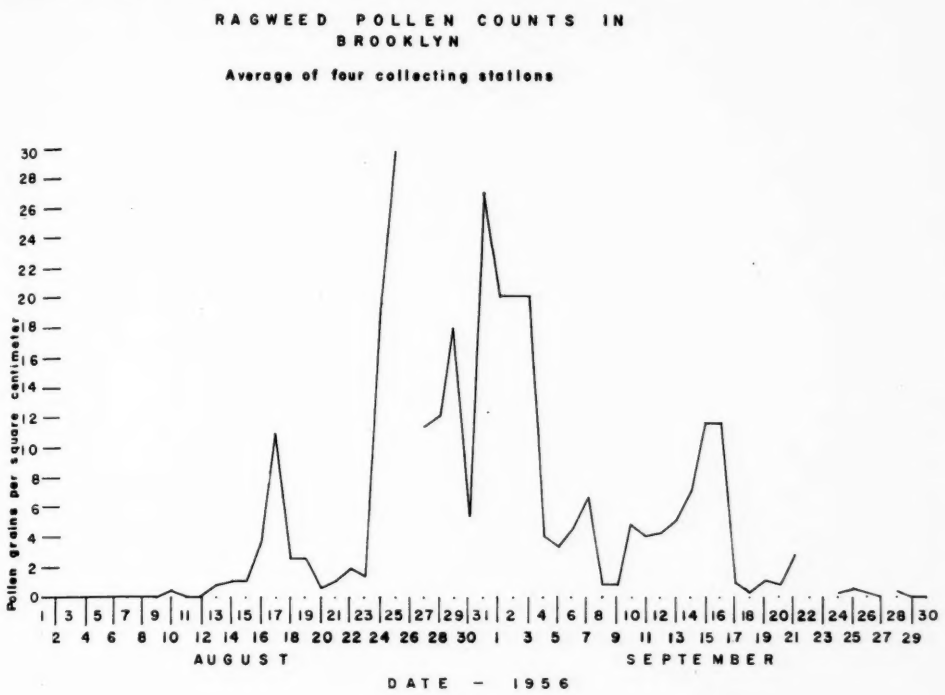


Fig. 4.

It will be recalled that the major purpose of this study was to determine whether an intensive program of ragweed eradication in the Bronx would reduce atmospheric pollen concentrations in that borough to levels lower than those prevailing in boroughs in which only partial programs of ragweed eradication were carried out. Since the borough of Manhattan had very little ragweed acreage, all of which was treated, the question to be answered was whether ragweed pollen concentrations were any lower in the Bronx, where 97 per cent of the ragweed acreage was treated, than in Brooklyn or Queens, where 6 per cent and 31 per cent of the ragweed acreage, respectively, were subjected to chemical treatment.

Average daily pollen counts in the Bronx and in Brooklyn were compared. Pollen counts were not made in Brooklyn on three of the sixty-one days of the study. Among the remaining fifty-eight days, there were eleven days on which the average pollen counts were the same in both boroughs, twenty-one days on which the counts in the Bronx were lower than those in Brooklyn, and twenty-six days on which the Bronx had higher pollen counts than Brooklyn. If there were no real differences between the boroughs, then the count in the Bronx

would be expected to be higher than that in Brooklyn on half of the days and lower on the other half, with deviation from this exact division on a chance basis. The 26 to 21 ratio actually found falls within this range of chance deviation, so that it can be said that the Bronx did not have lower or higher ragweed pollen counts than Brooklyn during August and September, 1956.

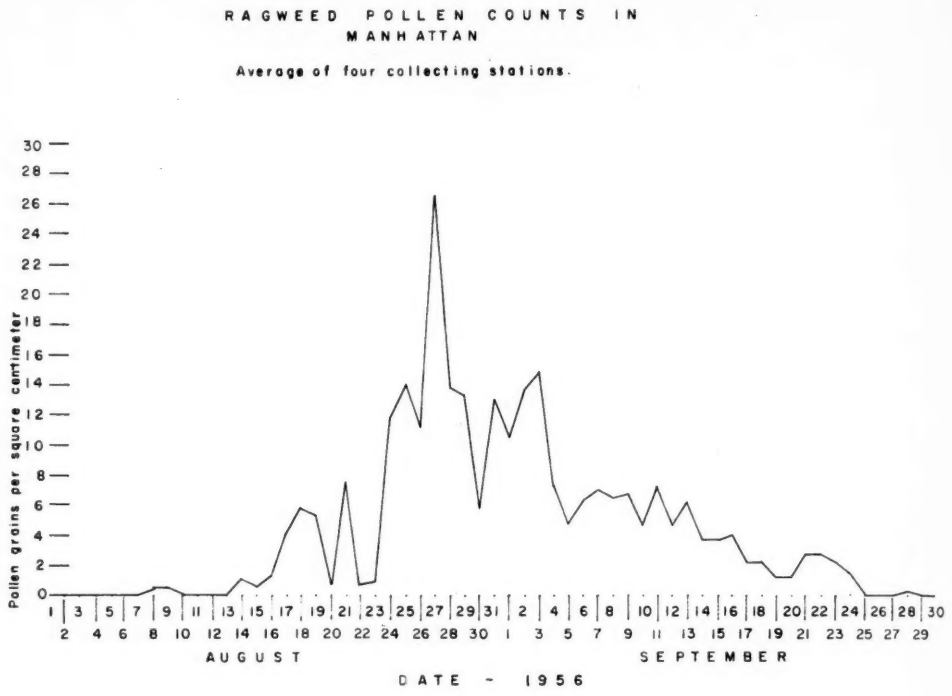


Fig. 5.

Because in this analysis of the total two-month period many days were included at both the beginning and the end of the period on which pollen counts were either 0 or very low, it was thought desirable to make another comparison between the boroughs for the period of moderate to high pollen counts, namely, August 17 to September 16. In this comparison, information was available for thirty days; no counts had been done in Brooklyn on one day. On nineteen days Bronx counts were higher than those in Brooklyn, and on eleven days they were lower. This analysis of the days during the height of the ragweed season yields the same results as the consideration of the total two-month period, namely, there is no evidence to show that the Bronx had consistently lower daily pollen counts than Brooklyn during the 1956 ragweed season.

A similar comparison was made between the Bronx and Queens. For the two-month period, the counts were the same on ten days; the Bronx had lower counts than Queens on twenty-six days and higher counts on twenty-five days. In the thirty-one days between August 17 and September 16, the Bronx had

lower counts than Queens on fourteen days and higher counts on seventeen days. As was the case in the comparison between the Bronx and Brooklyn, the data did not reveal consistently lower daily pollen counts in the Bronx than in Queens.

An interesting finding, for which we have no ready explanation, is that the comparison between daily pollen counts in the Bronx and Manhattan revealed a significant excess of days with high pollen counts in the Bronx. The

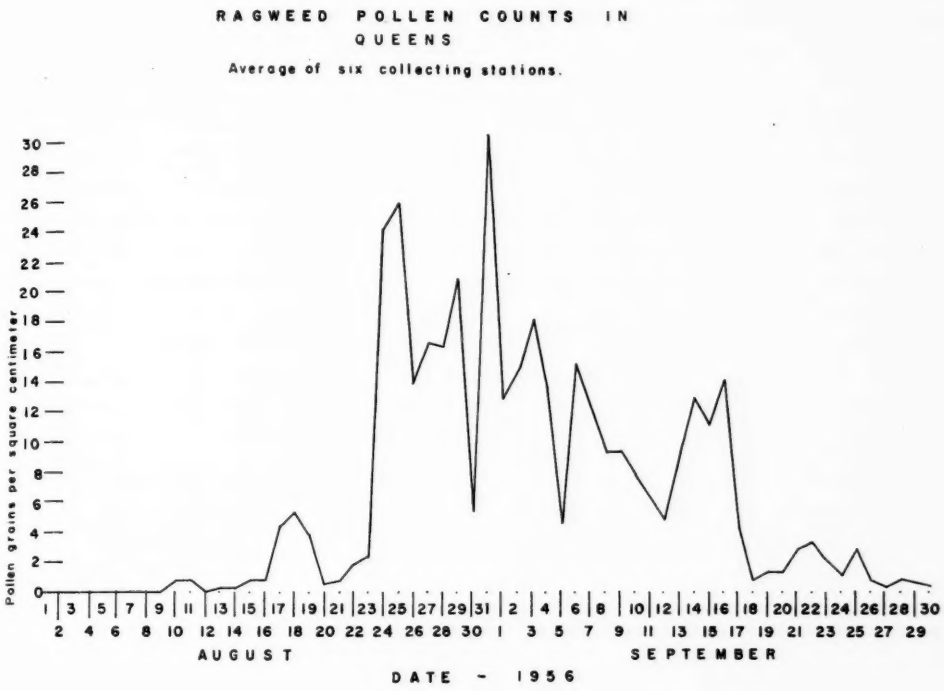


Fig. 6.

entire two-month period showed the same counts in both boroughs on eleven days, lower counts in the Bronx than in Manhattan on seventeen days, and higher counts on thirty-three days. Analysis of the restricted period from August 17 to September 16 showed a comparable significant difference: the Bronx had lower counts than Manhattan on eight days and higher counts on twenty-three days.

A comparison of Queens and Manhattan yielded significant findings of a similar nature. In the sixty-one-day period, the counts were the same in both boroughs on nine days, lower in Queens on seventeen days, and higher on thirty-five days. During the thirty-one-day period at the height of the pollen season, Queens had lower counts on nine days and higher counts on twenty-two days.

However, no significant differences were found between Brooklyn and Manhattan. On just about half the days the counts in Brooklyn were higher and on the other half they were lower than in Manhattan.

SUMMARY AND CONCLUSIONS

A study of ragweed hay fever was conducted by the Department of Health of the City of New York during the summer of 1956 to determine whether a borough-wide program of ragweed eradication would reduce atmospheric pollen concentrations in that borough to lower levels than those in the other boroughs not similarly treated. Ninety-seven per cent of the ragweed acreage in the Bronx, the experimental borough, was treated chemically.

An analysis of the pollen counts at a number of collecting stations in New York City (excluding Richmond) led to the following findings and conclusions:

1. The program of almost complete ragweed eradication in the Bronx did not result in lower daily pollen counts than in other boroughs. This study was not designed to, and did not, answer the question of whether the eradication program produced lower atmospheric pollen concentrations in the Bronx than would have occurred without such a program. It did reveal that, whether or not they were lowered, atmospheric pollen concentrations in the Bronx were still of the same order of magnitude as those prevailing in the boroughs of Brooklyn and Queens, where only "token" programs of ragweed eradication were carried out.

2. Daily pollen counts in New York City (excluding Richmond) were not significantly higher on the days when the prevailing winds came from the direction of neighboring land areas rather than from the Atlantic Ocean. This finding, based on the limited experience of one ragweed season, cannot be considered conclusive. It is contrary to the experience of others and inconsistent with the generally accepted theory that, even in an area where ragweed is generally prevalent, atmospheric concentrations of pollen are nonetheless materially increased by wind-borne pollen from neighboring ragweed-bearing land areas.

The Effect of a Ragweed Pollen Control Program on Hay Fever

Edward M. Cohart, M.D., New Haven, Conn., and Roscoe P. Kandle, M.D., New York, N. Y.

A STUDY of ragweed hay fever was conducted by the Department of Health of the City of New York during the summer of 1956. This study was made in an attempt to determine (1) whether a borough-wide program of ragweed eradication would result in significantly lower ragweed pollen counts in the treated borough than in other boroughs and (2) whether the ragweed hay fever patients in the treated borough would experience fewer or less severe symptoms than their counterparts in other boroughs. A discussion of the results as they bear upon the first question appeared in a previous article.[1]

METHOD

The study design called for an intensive program of ragweed eradication in one borough of New York City, with token programs in the remaining boroughs, and the comparison of hay fever symptoms during August and September among a group in the experimental borough with symptoms in similar groups in other boroughs. In order that any possible psychological effects of the knowledge of this program upon hay fever symptoms might be avoided, information about the intensive program in one borough was not publicized and the partial programs carried on in other boroughs in previous years were more or less continued.

The City of New York consists of five boroughs: the Bronx, Brooklyn, Manhattan, Queens, and Richmond. The borough of Richmond was eliminated from the study because of insufficient data. The Bronx was chosen as the experimental borough.

On the basis of surveys of the Bronx, Manhattan, and Queens, and a partial survey of Brooklyn, it was estimated that the Bronx had 2,000 acres of potential ragweed, Brooklyn had 2,500 acres, Manhattan had 35 acres, and Queens had 2,660 acres. In the 1956 ragweed elimination campaign, which began on June 20, 1,932 acres of ragweed in the Bronx were treated chemically with 2, 4-D, accounting for 96.6 per cent of this borough's ragweed acreage. In Brooklyn only 141 acres, or 5.6 per cent of its 2,500 acres of ragweed, were sprayed. In Manhattan 100 per cent of the 35 acres of ragweed were sprayed. In Queens 833 acres were sprayed, representing 31.3 per cent of the 2,660 acres of ragweed in that borough.

From the Department of Public Health, Yale University, and the Department of Health of the City of New York.

Received for publication Dec. 2, 1958.

Symptoms of hay fever were recorded by the volunteer participants in this study in a "hay fever diary" made available by the Department of Health. The diary, a four-page printed questionnaire, made provision for check-mark entries for every day of the months of August and September, indicating whether the participant was in the city on the day and question and whether he had no symptoms or mild, moderate, or severe symptoms of hay fever. Each participant was also requested to report his name, address, place of work, and type of work and to indicate whether he had air conditioning at home or at work. In addition, he was asked whether he had had treatment for hay fever in 1956, whether he was taking drugs for his hay fever in 1956, and whether his hay fever in 1956 was better or worse than or the same as in 1955.

TABLE I. CLASSIFICATION OF 855 HAY FEVER DIARIES

BOROUGH	DISCARDED DIARIES (1)	(2)	(3)	(4)	(5)	TOTAL	CODED DIARIES FOR TABULATION	TOTAL
Bronx	38	4	0	7	—	49	167	216
Brooklyn	58	9	3	10	—	80	171	251
Manhattan	41	7	3	6	—	57	151	208
Queens	42	6	2	5	—	55	107	162
Richmond	—	—	—	—	18	18	0	18
Totals	179	26	8	28	18	259	596	855

(1) = Asthma or hay fever before ragweed season.
(2) = Diary cannot be interpreted.
(3) = Out of city for almost entire period of diary.
(4) = No symptoms of hay fever during ragweed season.
(5) = Sample too small.

The cooperation of the New York Allergy Society and the Department of Hospitals of New York City was enlisted for the distribution of the diaries to patients.

CHARACTERISTICS OF THE SAMPLE

It was anticipated that a fairly large sample of hay fever patients in New York City would make it possible to compare the ragweed hay fever group in the Bronx with the groups in other boroughs for the variable of ragweed eradication only, on the theory that the several uncontrolled variables would tend to be equalized for each borough in a large sample. It should be emphasized, however, that it was recognized from the very beginning of this study that the anticipated sample was not likely to be random and that no means were at hand to make it representative, since the factors of self-selection operating among the volunteers in this study were not known. Therefore, the data cannot be interpreted as indicative of prevalence or of any other characteristic of the totality of hay fever sufferers in any borough of New York City or in the city as a whole.

Unfortunately, the number of persons participating in the study was not nearly so large as had been expected in light of the efforts made to enlist the cooperation of the city's practicing allergists through the New York Allergy Society. Nevertheless, a sufficient number of diaries was collected to make certain borough comparisons possible.

Several thousand diaries were distributed to physicians and clinics, but it is not known how many were actually given to patients. Eight hundred fifty-five diaries were returned to the Department of Health. Two hundred fifty-nine were not used in this analysis for the reasons indicated in Table I. The major reason for exclusion was the presence of asthma or hay fever of five or more days' duration before the onset of the ragweed season. While it is realized that an unknown number of patients included in the study also probably suffered from other allergies in addition to their ragweed hay fever, elimination of all patients with multiple allergies was not practicable. However, it was felt that exclusion of the group with manifest nonragweed symptoms would serve to make the remaining sample a more delicate indicator of the effects of environmental control on ragweed hay fever.

Five hundred ninety-six diaries were used in the analysis: 167 in the Bronx, 171 in Brooklyn, 151 in Manhattan, and 107 in Queens. If information had been available for every day during August and September for each one of these 596 persons, there would have been 36,356 patient-days of observation. However, no information was recorded for 10.3 per cent of these patient-days. Variation among the boroughs in respect to completeness of reporting was small; there was no information for 9 per cent of all patient-days in Queens, 10 per cent in the Bronx, and 11 per cent in Brooklyn and Manhattan (Table II).

TABLE II. AVERAGE PERCENTAGE, BY BOROUGH, OF PATIENTS WITH NO INFORMATION ON ANY ONE DAY

DATES	PER CENT WITH NO INFORMATION				
	BRONX (167 PATIENTS)	BROOKLYN (171 PATIENTS)	MANHATTAN (151 PATIENTS)	QUEENS (107 PATIENTS)	FOUR BOROUGHS (596 PATIENTS)
August					
1-10	27.0	37.7	30.6	26.2	30.8
11-20	14.9	16.7	17.7	15.2	16.2
21-31	5.3	3.6	6.3	2.8	4.6
September					
1-10	2.1	1.5	3.1	3.0	2.4
11-20	3.3	2.8	3.2	3.3	3.1
21-30	6.2	5.0	5.3	4.4	5.3
All 61 days	9.7	11.1	11.0	9.0	10.3

A marked temporal trend was found in completeness of reporting. This trend was essentially similar in each of the boroughs. For the city as a whole, no data were obtained for 31 per cent of the patient-days from August 1 to August 10, 16 per cent from August 11 to August 20, 5 per cent from August 21 to August 31, and 2 to 5 per cent for the three ten-day periods in September. Borough differences were not significant.* It seems reasonable to assume that the high proportion of absent entries in the diaries in the first two ten-day periods in August probably resulted from the practice of many patients of not beginning their diary entries until they began to have symptoms. In the absence of evidence in support of this contention, however, we followed the conservative approach of eliminating these days from the analysis of the data.

*A difference was considered to be statistically significant if it could have occurred by chance less than one in 20 times ($P < .05$).

Almost 9 per cent of the participants were out of the city on any one day during August and September. This percentage, at its highest during the first ten days of August when almost 15 per cent of the participants were out of the city on any one day, dropped gradually during succeeding ten-day periods in August to about 10 per cent in the first ten days in September. The succeeding two ten-day periods in September showed 3 and 2 per cent, respectively, out of the city. Table III shows that whereas the percentage out of the city on any one day was quite similar for the samples in the boroughs of the Bronx, Manhattan, and Queens, Brooklyn had consistently lower values than those of the other three boroughs, with the exception of the period of September 21 to 30. Nevertheless, the difference between Brooklyn and the other three boroughs, when tested statistically in terms of the number of persons in each sample, was not significant.

TABLE III. AVERAGE PERCENTAGE OF PATIENTS, AMONG THOSE WITH INFORMATION, OUT OF CITY ON ANY ONE DAY, BY BOROUGH

DATES	PER CENT OUT OF CITY				
	BRONX	BROOKLYN	MANHATTAN	QUEENS	FOUR BOROUGHS
August					
1-10	15.4	8.8	15.6	20.1	14.6
11-20	14.0	9.5	14.9	13.8	12.9
21-31	14.6	7.4	12.2	14.7	11.9
September					
1-10	13.1	5.6	9.4	12.0	9.8
11-20	2.8	1.4	2.9	5.3	2.9
21-30	2.4	2.2	1.8	1.9	2.1
All 61 days	10.2	5.6	9.0	11.0	8.7

Forty-five per cent of the participants in the study were male. The borough of Queens, with 56 per cent male, differed significantly from the other three boroughs in which 43 per cent were male. The fact that in Queens a significantly higher proportion of participants were white-collar workers who worked in a borough other than the one in which they lived was probably related to the significantly higher proportion of males in the Queens sample.

Ten per cent of the participants stated that they had some kind of air conditioning at home, and 20 per cent had some kind of air conditioning at work. The percentage with air conditioning either at home or at work did not vary significantly among the groups in the four boroughs.

Sixty-two per cent of the participants said that they were taking one medication or another for their hay fever during the 1956 season. Borough differences, a range of from 57 per cent in Manhattan to 67 per cent in Queens, were not statistically significant.

From the standpoint of comparability, undoubtedly the most important statistically significant difference among the boroughs was in the percentages who said that they had had any kind of treatment for hay fever in 1956. Sixty-six per cent of all the participants said that they had had treatment for hay fever in 1956. The percentages in Manhattan and Queens were about the same as for the city as a whole. However, the Bronx, with 53 per cent treated, had

a significantly lower rate, and Brooklyn, with 79 per cent treated, had a significantly higher rate treated than did the other boroughs. This important difference in treatment among the samples by borough must be given proper consideration in the interpretation of the results of this study.

One further comment should be made about the sample before the results are reported. The analysis of symptoms was performed for each borough on a daily basis, the actual number of persons in town for whom information was available on any given day being used as the population at risk. Because of the high proportion of "no entries" early in August and the larger numbers out of the city at this time, the patient-days of observation increased from a low of 59 per cent of the theoretical maximum in the first ten days of August to 94 per cent during the middle ten days of September (Table IV). The possible effect of this increase on the findings will be considered in the interpretation of the results.

TABLE IV. AVERAGE PERCENTAGE OF PATIENTS IN CITY WITH INFORMATION AS TO SYMPTOMS ON ANY ONE DAY, BY BOROUGH

DATES	PER CENT IN CITY WITH INFORMATION				
	BRONX (167 PATIENTS)	BROOKLYN (171 PATIENTS)	MANHATTAN (151 PATIENTS)	QUEENS (107 PATIENTS)	FOUR BOROUGHS (596 PATIENTS)
August					
1-10	61.8	56.8	56.8	59.0	59.1
11-20	73.2	75.4	70.1	73.1	73.0
21-31	80.9	89.2	82.3	82.9	84.0
September					
1-10	85.1	93.0	87.8	85.4	88.1
11-20	94.0	95.9	94.0	91.6	94.1
21-30	91.6	92.9	93.0	93.8	92.7
All 61 days	81.1	84.0	81.0	81.0	81.9

RESULTS

For the city as a whole, in the two-month period from August 1 to September 30, 1956, hay fever symptoms of any degree of severity were reported on 62 per cent of the patient-days on which the participants were in the city and for which information was furnished (Table V). The percentage of patient-days with symtoms of hay fever during this two-month period was not statistically significantly different in any of the four boroughs from that of the city as a whole. Sixty-four per cent of the patient-days in the Bronx and Brooklyn, 62 per cent in Manhattan, and 58 per cent in Queens were marked by symptoms.

Inasmuch as it was shown previously that the samples in the four boroughs did not differ significantly in respect to the proportions in the city and giving information during any one of the six time periods studied, the boroughs are comparable on that score. It can be said, therefore, that no significant differences in the percentage of patient-days with symptoms during the two months of the study were found among the four boroughs.

The percentage of days with symptoms rose from a base line of 6 during the first ten days of August (this base line presumably due to allergies other than ragweed) to a high of 82 during the first ten days of September. The start of

the ragweed season during the second ten days of August caused the average number of patient-days with symptoms of hay fever to rise to 38 per cent. This percentage increased to 73 during the last eleven days of the month and, as

TABLE V. PERCENTAGE OF PATIENT-DAYS WITH SYMPTOMS OF HAY FEVER

DATES		PATIENT-DAYS OF EXPOSURE	PATIENT-DAYS WITH SYMPTOMS		PATIENT-DAYS WITH MODERATE OR SEVERE SYMPTOMS		PATIENT-DAYS WITH SEVERE SYMPTOMS	
			NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Bronx (167 coded diaries)								
August	1-10	1,032	72	7.0	14	1.4	9	0.9
	11-20	1,222	501	41.0	141	11.5	38	3.1
	21-31	1,486	1,141	76.8	568	38.2	235	15.8
September	1-10	1,421	1,215	85.5	763	53.7	337	23.7
	11-20	1,569	1,304	83.1	711	45.3	249	15.9
	21-30	1,529	1,054	68.9	461	30.2	142	9.3
August and September		8,259	5,287	64.0	2,658	32.2	1,010	12.2
Brooklyn (171 coded diaries)								
August	1-10	972	59	6.1	7	0.7	5	0.5
	11-20	1,289	491	38.1	130	10.1	45	3.5
	21-31	1,678	1,207	71.9	599	35.7	162	9.6
September	1-10	1,590	1,319	83.0	772	48.6	309	19.4
	11-20	1,640	1,356	82.7	692	42.2	245	14.9
	21-30	1,588	1,129	71.1	489	30.8	151	9.5
August and September		8,757	5,561	63.5	2,689	30.7	917	10.5
Manhattan (151 coded diaries)								
August	1-10	885	71	8.0	13	1.5	6	0.7
	11-20	1,058	430	40.6	154	14.6	63	6.0
	21-31	1,367	1,013	74.1	540	39.5	262	19.2
September	1-10	1,326	1,050	79.2	666	50.2	317	23.9
	11-20	1,420	1,101	77.5	629	44.3	259	18.2
	21-30	1,405	971	69.1	402	28.6	190	13.5
August and September		7,461	4,636	62.1	2,404	32.2	1,097	14.7
Queens (107 coded diaries)								
August	1-10	631	24	3.8	4	0.6	3	0.5
	11-20	782	240	30.7	58	7.4	20	2.6
	21-31	976	649	66.5	306	31.4	113	11.6
September	1-10	914	737	80.6	433	47.4	162	17.7
	11-20	980	790	80.6	411	41.9	141	14.4
	21-30	1,004	635	63.2	252	25.1	58	5.8
August and September		5,287	3,075	58.2	1,464	27.7	497	9.4
Four Boroughs (596 coded diaries)								
August	1-10	3,520	226	6.4	38	1.1	23	0.6
	11-20	4,351	1,662	38.2	483	11.1	166	3.8
	21-31	5,507	4,010	72.8	2,013	36.6	772	14.0
September	1-10	5,251	4,321	82.3	2,634	50.2	1,125	21.4
	11-20	5,609	4,551	81.1	2,443	43.6	894	15.9
	21-30	5,526	3,789	68.6	1,604	29.0	541	9.8
August and September		29,764	18,559	62.4	9,215	31.0	3,521	11.8

previously stated, to a high of 82 during the first ten days of September. The second ten days of September saw an almost imperceptible fall to 81 per cent, while symptoms were present on 69 per cent of the patient-days observed during the last ten days of the month.

It can be seen in Table V that for each of the six time periods the boroughs vary somewhat about the mean values for the city as a whole. However, all of these differences fall within the range of chance variation. Therefore, for each of the six time periods, as for the two-month total, no significant differences were observed among the boroughs in the percentage of patient-days with symptoms.

Conceivably, a program of ragweed elimination might alter the severity rather than the frequency of symptoms. Table V reveals that 31 per cent of the patient-days in the city as a whole were characterized by either moderate or severe symtoms of hay fever and 12 per cent by severe symptoms only. Both of these classes of severity of symptoms revealed the same temporal trends as were shown previously for symptoms of all degrees of severity, the high mark being reached during the first ten days of September.

A comparison by borough of the percentage of patient-days marked by moderate symptoms during August and September and for each of the six time periods failed to reveal statistically significant differences. A comparison of the percentage of patient-days with severe symptoms yielded similar results, with two exceptions. In one case, during the period of August 21 to 31, severe symptoms occurred in 19.2 per cent of the patient-days in Manhattan as compared with 9.6 per cent in Brooklyn. In the second case, 13.5 per cent of the patient-days in Manhattan from September 21 to September 30 were characterized by severe symptoms as compared with 5.8 per cent in Queens. There appears to be no ready explanation for either of these differences. It should be remembered, however, that the 5 per cent level of probability was employed as the criterion of statistical significance in this study and that, under these circumstances, a so-called "significant" result might be expected on a *chance basis alone* in one of every twenty comparisons on the average. Therefore, the two differences found may be chance occurrences and not real differences. In any event, neither of these differences involves the borough of the Bronx, in which the intensive program of ragweed elimination was undertaken. Neither frequency of all symptoms nor of moderate or severe symptoms differed significantly in the Bronx from any of the other three boroughs.

It will be recalled, however, that significantly fewer patients (53 per cent) in the Bronx and significantly more (79 per cent) in Brooklyn than in the other boroughs claimed to have had treatment for hay fever. It might be expected, therefore, that, had the percentage receiving treatment been the same in the Bronx and Brooklyn as in Manhattan and Queens, the Bronx sample would have had less frequent and less severe symptoms than were actually experienced, while symptoms in the Brooklyn group would perhaps have been more frequent and more severe. This contention cannot be refuted from the available evidence.

However, there are two observations that would seem to indicate that this difference in percentage of patients in the Bronx and Brooklyn who had received treatment does not invalidate the finding that a localized program of almost complete ragweed elimination did not materially affect the frequency or severity of hay fever symptoms. The ragweed pollen counts in the Bronx, where an intensive program of ragweed elimination was carried out, and in Manhattan,

which because of its almost complete use of land for buildings is practically free of indigenous ragweed, were not significantly lower than those for Brooklyn and Queens. Therefore, this localized program of ragweed pollen elimination, since it did not materially influence the pollen counts, would not be expected to influence hay fever symptoms.

If the program of almost complete ragweed elimination in the Bronx had been of benefit to hay fever patients in that borough, then a larger percentage of the Bronx study group than of the groups in other boroughs should have felt better during the 1956 ragweed season than they did during the 1955 season.

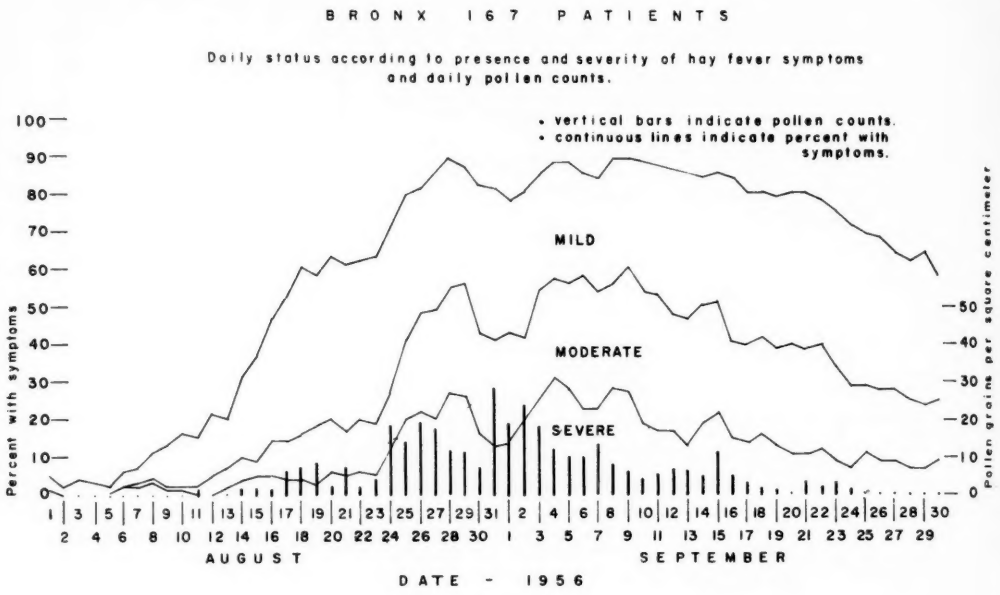


Fig. 1.

This was not the case. Sixty-six per cent of all the patients answering this question said that they felt better in the 1956 than in the 1955 ragweed season, 21 per cent said that they were the same, and 13 per cent said that they were worse. The variations by borough were not statistically significant. Sixty-four per cent of the groups in Brooklyn and Manhattan, 68 per cent in the Bronx, and 70 per cent in Queens said that they were better in 1956 than in 1955, while 12 per cent of those in the Bronx and Manhattan, 13 per cent of the group in Brooklyn, and 15 per cent of the group in Queens said that they were worse in 1956 than in 1955.

The results of this study of ragweed hay fever symptoms are portrayed graphically for each borough and for the city, excluding Richmond, in Figs. 1 through 5. These graphs, in addition to showing the comparability of symptoms with regard to both frequency and severity that has just been discussed, reveal several other interesting findings.

In the first place, the graphs show the general similarity of pollen counts in the four boroughs, a finding discussed in detail in a previous publication,[1] in

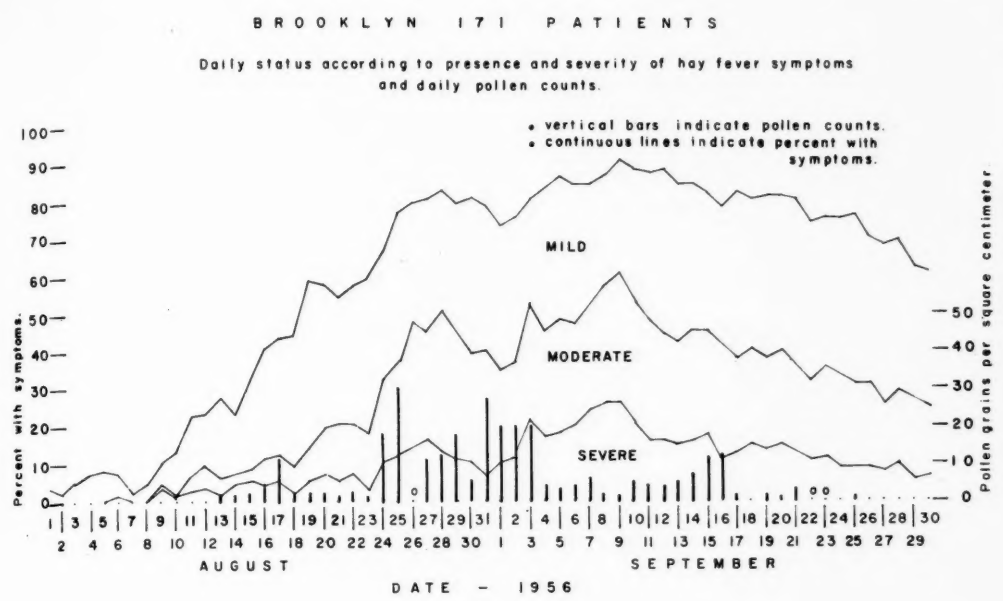

BROOKLYN 171 PATIENTS
Daily status according to presence and severity of hay fever symptoms and daily pollen counts.
• vertical bars indicate pollen counts.
• continuous lines indicate percent with symptoms.
MILD
MODERATE
SEVERE
Percent with symptoms.
Pollen grains per square centimeter
AUGUST
SEPTEMBER
DATE - 1956

Fig. 2.

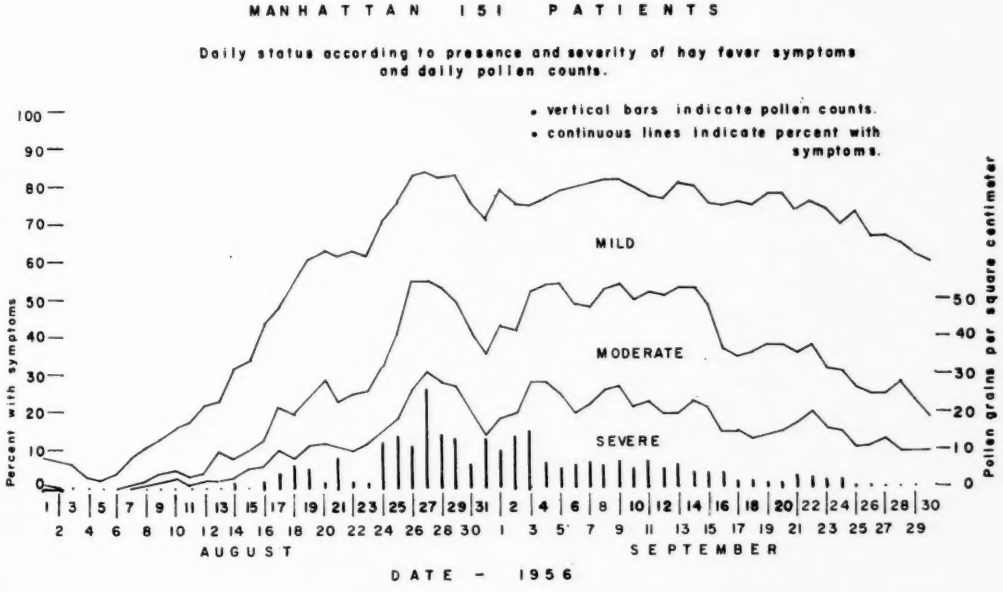

MANHATTAN 151 PATIENTS
Daily status according to presence and severity of hay fever symptoms and daily pollen counts.
• vertical bars indicate pollen counts.
• continuous lines indicate percent with symptoms.
MILD
MODERATE
SEVERE
Percent with symptoms
Pollen grains per square centimeter
AUGUST
SEPTEMBER
DATE - 1956

Fig. 3.

which it was shown that pollen counts were not significantly lower in the Bronx than in Brooklyn, Manhattan, or Queens. Thus, despite the intensive program of ragweed elimination in the Bronx, pollen counts were not lower and symptoms were not less frequent or severe in this borough.

While the greatest frequency and severity of symptoms occurred during the period of high pollen counts, the rise in frequency and severity of symptoms

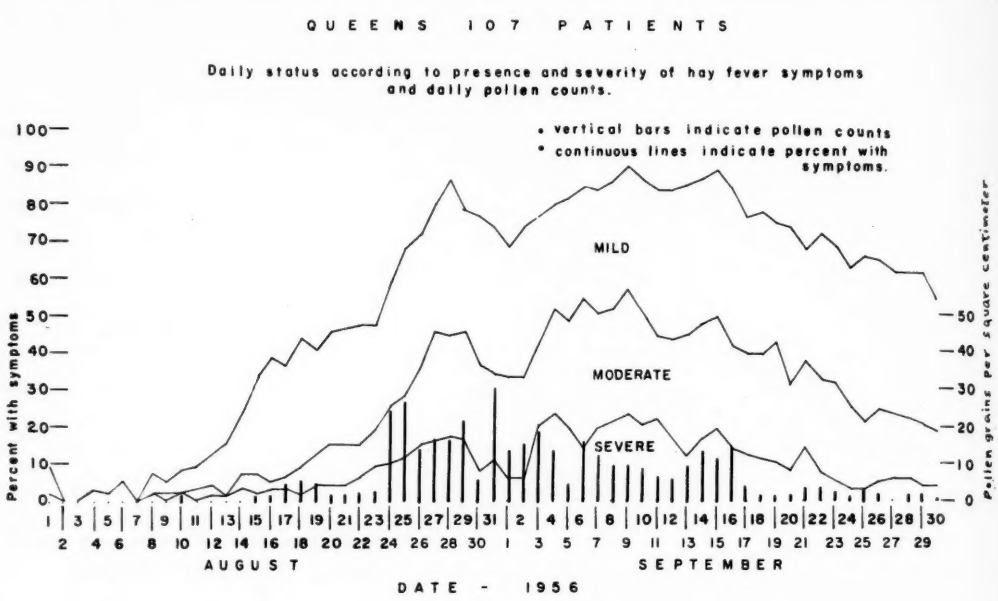


Fig. 4.

commenced prior to the middle of August, when appreciable quantities of ragweed pollen first began to be detected. Equally interesting is the observation that more than 60 per cent of the patients still continued to have hay fever symptoms during the last five days of September when no appreciable amounts of ragweed pollen could be detected in the atmosphere.

It will be recalled that almost 15 per cent of the participants in this study were out of the city on any one day early in August as compared with 2 per cent at the end of September. If the assumption is made that all of these persons would have shown symptoms had they been in the city and made entries in their diaries, then 20 per cent would have had symptoms during the first ten days of August and slightly less than 70 per cent during the last ten days of September. If the even more unlikely assumption is made that, in addition, all patients who failed to make an entry in their diary on any one day had hay fever symptoms on that day, then 45 per cent would have had symptoms during the first ten days of August as compared with 71 per cent during the last ten days of September. In all probability these assumptions are completely unwarranted, but even if they were true the resulting findings would still present

the picture of a much greater frequency of hay fever symptoms, in the absence of recoverable amounts of ragweed pollen, at the end of September than at the beginning of August.

Neither the preseasonal rise nor the persistence of symptoms at the end of the season has a ready explanation. It may be that a higher mold spore content of the atmosphere during these periods, as recently reported by Merksamer and Sherman,[2] accounts for this finding. Or possibly the increase in symptoms predating the ragweed season can be explained, at least in part, on a psychological basis of anticipation, while the high postseasonal incidence of symptoms may be the result of sensitization, either physiological or psychological.

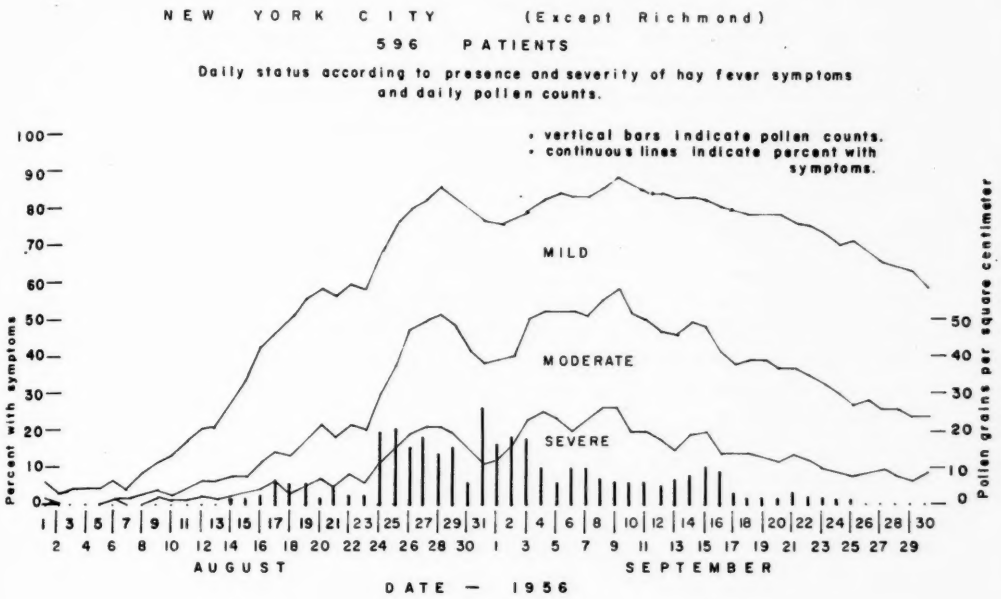


Fig. 5.

SUMMARY AND CONCLUSIONS

A study of ragweed hay fever was conducted by the Department of Health of the City of New York during the summer of 1956 to determine whether a borough-wide program of ragweed eradication would reduce the frequency or severity of symptoms among presumed ragweed hay fever patients in the treated borough.

More than 95 per cent of the 2,000 acres of ragweed in the Bronx were eliminated by chemical treatment. Patients in this as well as other boroughs were asked to record symptoms daily during August and September in a hay fever diary. The difficulties encountered in the study and the characteristics of the sample are discussed. The sample was neither random nor representative, so that no conclusions can be drawn concerning the total picture of ragweed hay fever in New York City.

Despite the difficulties inherent in an epidemiological study of this nature, the following conclusions are warranted:

1. A localized program of almost complete ragweed elimination in the Bronx did not result in that borough's having significantly lower pollen concentrations or a significantly lower frequency of severe symptoms or of all symptoms than did the boroughs of Brooklyn and Queens.

2. Manhattan, which because of the almost complete use of its land for buildings is practically free of indigenous ragweed, also did not have significantly lower pollen concentrations or fewer days of severe symptoms or of all symptoms than did the other boroughs.

3. It appears, therefore, that ragweed pollen concentrations in New York City and the symptoms of hay fever resulting therefrom are primarily dependent on pollen that is wind-borne from areas outside the city's boundaries; a total program of ragweed elimination in New York City would not succeed in reducing materially the symptoms of ragweed hay fever among the city's residents.

4. An increase in hay fever symptoms among the selected group of patients in this study antedated by about one week the finding of ragweed pollen in the atmosphere.

5. Hay fever symptoms persisted among more than half of this group of patients at the end of September in the absence of recoverable amounts of ragweed pollen in the atmosphere.

REFERENCES

1. Cohart, E. M., and Kandle, R. P.: The Effects of a Ragweed Control Program on Ragweed Pollen Counts, J. ALLERGY **30**: 287-298, 1959.
2. Merksamer, D., and Sherman, H.: An Evaluation of Atmospheric Mold Spores as Allergens in the New York Metropolitan Area, J. ALLERGY **29**: 60-71, 1958.

Immune Sera in the Study of Food Antigens

II. Crustaceans

Sheldon G. Cohen, M.D., and Charles B. Reif, Ph.D.,** Wilkes-Barre, Pa.*

INTRODUCTION

In a previous study[1] the potential value of immune rabbit sera in the study of component antigenic fractions of fish extracts and fish-derivative preparations was demonstrated. It was decided, therefore, to extend these experimental techniques to an investigation of some problems related to the crustaceans, including (1) cross-antigenicity between skin-test extracts of those shellfish (arthropods) responsible for allergic reactions in man, (2) antigenic relationships between taxonomically related "insect-type" (entomostracan) crustaceans and larger edible shellfish (malacostracans), and (3) uniformity of qualitative antigenic content of shellfish (crustacean) extracts prepared for skin-testing purposes in different laboratories.

MATERIALS AND METHODS

Extracts of the edible muscle of common commercially available crustaceans, including lobster (genus *Homarus*), crab (genus *Cancer*), shrimp (genus *Panilurus*) were prepared† by standard methods of extraction in glycerinated buffered saline. Similar extraction techniques were employed in our laboratory with abdominal muscles of fresh-water crayfish obtained on the open food market‡ and also collected by us from local lakes and streams.

Crustaceans of the "insect type" (entomostracan) employed were of two varieties. Water fleas (*Daphnia pulex*), taken with a fine-mesh net from a depth of 40 feet in a local oligotrophic lake between 10 A.M. and 2 P.M., were obtained in practically a pure collection. They were immediately frozen to prevent deterioration prior to extraction. Brine shrimp (*Artemia salina*),

From the Department of Biology, Wilkes College.

Presented in part at the fifteenth annual meeting of the American Academy of Allergy, Chicago, Illinois, Feb. 9 to 11, 1959.

This study was sponsored by the Committee on Food Allergy of the Research Council, American Academy of Allergy.

Received for publication Dec. 31, 1958.

*Associate Professor of Biological Research.

**Professor of Biology.

†These extracts were prepared and supplied through the courtesy of Margaret Strauss, Director of the Allergy Laboratory, University Hospital, New York University—Bellevue Medical Center.

‡Supplied through the courtesy of Dr. Vincent J. Derbes, Tulane University School of Medicine, New Orleans, Louisiana.

gathered from Great Salt Lake in a pure collection, were shipped to us in the frozen state from Utah.* Whole-body extracts of these tiny crustaceans (phyllopods) were prepared in buffered saline immediately upon thawing.

Immunizing dosages and schedules for the preparation of rabbit antisera and the agar-gel diffusion precipitin tests (Ouchterlony technique) for qualitative antigenic analyses were identical with those previously reported for the study of fish extracts.[1]

RESULTS

The results of multiple agar-gel diffusion precipitin tests employing each crustacean extract with each homologous and heterologous antiserum are summarized in Table I.

TABLE I

	NUMBER OF PRECIPITIN BANDS ANTISERA TO:						
EXTRACT	LOBSTER	CRAB	SHRIMP	ROCK LOBSTER	CRAW-FISH	DAPHNIA	BRINE SHRIMP
Lobster	6-8	3	4	2	2	0	1
Crab	4	7-9	4	1	2	0	0
Shrimp	2-4	3-4	6	1	1	0	1
Rock lobster	2	1	3	5-7	2	0	1
Crayfish	3	3	2	3	4	2	2
Daphnia	0	0	0	0	1	6-7	1
Brine shrimp	1	0	1	1-2	1	1	3-4

The results of agar-gel diffusion precipitin tests employing randomly selected samples of skin-test crustacean extracts prepared in different laboratories, and presumably by the same methods, with homologous species antisera to the crustaceans are summarized in Table II.

TABLE II

		NUMBER OF PRECIPITIN BANDS			
ANTISERUM TO:	+ EXTRACT	A	B	C	D
Crab	Crab	7-9	8-9	0	0
Lobster	Lobster	6-7	1	5	
Shrimp	Shrimp	6	0-1	2-3	

COMMENT

The multiplicity of distinct antigenic components[4-9] within the edible muscles of crustaceans has been demonstrated by their precipitin-producing properties in rabbits. It is to be pointed out that these figures may not include the total number of potential antigens for either lobster, crab, shrimp, rock lobster, or crayfish, for the respective immunizing extracts did not include either related blood hemocyanins[2-3] or hepatopancreatic organs. Among the five common groups studied, only a partial degree of cross-antigenicity could be noted with these in vitro techniques. No more than two-thirds of

*Collected by and supplied through the courtesy of Mr. C. C. Saunders of Odgen, Utah.

the total antigenic fractions identifiable in any one species were demonstrated in reactions of identity, and in most instances the number of antigens common to any two species of crustaceans was found to be considerably less. Furthermore, in searching for reactions of identity between component antigens of more than two species, it was impossible to find bands of precipitate common to the five representative crustaceans studied. Thus, even though the taxonomic classification (Table III) of the class *Crustacea* places lobster, shrimp, crab, rock lobster, and crayfish in the same order, a relationship that might be characterized by a common antigenic denominator for their muscle proteins could not be established. Such a relationship could explain variations in uniformity of skin-test patterns and clinical manifestations of hypersensitivity. Determinants of such reactions would then include not only a quantitative factor of group antigen[4] but also variations in content of qualitatively distinct serum reagins to species-specific antigens in crustacean-allergic patients. At this stage, however, the evidence for relating allergenic constituents of a food or food extract to its precipitin-producing antigenic components can be considered only presumptive.

TABLE III. CLASSIFICATION OF CRUSTACEANS INCLUDED IN THIS STUDY

Phylum Arthropoda	
Class Crustacea	
Subclass Entomostraca	
Order Branchiopoda	
Suborder Phyllopoda	*Artemia salina*
Suborder Cladocera	*Daphnia pulex*
Subclass Malacostraca	
Division Eumalacostraca	
Order Decapoda	
Genus Homarus	lobster
Genus Peneus	commercial shrimp
Genus Panilurus	rock lobster
Genus Cambarus	fresh-water crayfish
Genus Cancer	crab

The importance of insect allergy is receiving increasing attention. The current status of this intriguing subject has been recently reviewed and summarized by Perlman.[5] It is to be noted that organisms placed in the classes *Crustacea* and *Insecta* are collectively members of the same phylum (*Arthropoda*) and that entomostracans (daphnia and brine shrimp), because of their small size, have often been too loosely categorized as "insects." Therefore, a study of the antigenic relationship of daphnia and brine shrimp to larger edible shellfish appeared to be of more than academic interest in the consideration of crustacean allergy.

The usual diurnal habitat of daphnia in fresh-water lakes places them at greater depths than are likely to be encountered by the average swimmer. However, it is possible that they might come into contact with shellfish-allergic human beings in shallow ponds and drainage ditches or by the inhalation of daphnia "dust" resulting from the handling of compressed dried cakes of these organisms which are available commercially as a source of fish food. Instances of allergic reactions in such circumstances have been reported.[6-7] Frozen brine

shrimp have also been made available to tropical fish hobbyists, and Perlman[5] points out possible additional sources of contact to ocean swimmers and to residents in areas where the tiny eggs of the brine shrimp could conceivably be part of the atmospheric dust.

Among members of the order *Decapoda* (lobster, shrimp, rock lobster, crayfish) possessing multiple common antigens, only crab failed to exhibit cross-antigenicity with brine shrimp by the common bond of at least one antigenic fraction. *Daphnia pulex,* on the other hand, demonstrated antigenic constituents which were common only to brine shrimp and crayfish. Its closer antigenic relationship to brine shrimp might be suspected from their taxonomic relationship within the same order, *Branchiopoda* (Table III). It is also to be pointed out that, whereas extracts of daphnia and brine shrimp were prepared from whole organisms including their hemocyanins and hepatopancreatic organs, extracts of the larger crustaceans included only those antigens characteristic of edible abdominal muscle. The exact reason for selective cross-antigenicity of daphnia with only one member of the order *Decapoda* (crawfish) is not apparent. However, one might suspect this to exist on an ecologic basic, since they both are the only fresh-water crustaceans represented in this study. In further consideration of ecologic possibilities, it is of interest that the brine shrimp (*Artemia salina*) studied here evolved from fresh-water progenitors which were able to adapt to the increased salt content of the Great Salt Lake in Utah. That potential clinical allergy to daphnia and brine shrimp in shellfish-sensitive human beings may be related to a variable presence of reagins to specific crustacean antigenic fractions is further suggested by the studies of Perlman.[5, 8] Crab- and lobster-sensitive patients often reacted to skin testing with daphnia and brine shrimp extracts, but in a number of circumstances positive reactions were produced for one crustacean species and not for another.

The wide variation in content of precipitin-producing antigens demonstrated among species-identical crustacean extracts prepared in different laboratories resembles a similar situation previously noted among fish extracts.[1] Although the instability of shellfish extracts has been mentioned,[9] other possible factors under consideration and study by the Committee on Food Allergy of the Research Council of the American Academy of Allergy include the effect of preliminary freezing of the raw material, variations in technique of extraction and storage, and the addition of glycerin preservative. The importance of possible variations in qualitative antigenic content of shellfish extracts influencing results and interpretation of diagnostic skin tests is self-evident and a matter for our further attention and evaluation.

SUMMARY

1. Sera from specifically immunized rabbits were employed in agar-gel diffusion precipitin tests in the qualitative antigenic analyses of crustacean extracts.

2. Multiple antigenic components (4-9) were demonstrated in the edible abdominal muscles of lobster, crab, shrimp, lobster tail, and fresh-water crayfish and in the bodies of entomostracans (''insect type'' crustaceans), daphnia and brine shrimp.

3. Only a partial degree of qualitative cross-antigenicity was noted among various taxonomically related edible shellfish, and an antigen common to all five could not be demonstrated.

4. Partial qualitative cross-antigenicity was noted among taxonomically related entomostracans (''insect-type'' crustaceans), *Artemia salina* (brine shrimp) and *Daphnia pulex* (water fleas). Some antigenic components of brine shrimp were common to those of the edible malacostracans (shellfish), whereas daphnia contained antigen common only to ecologically related fresh-water crayfish.

5. A wide qualitative variation among individual component antigens of crustaceans was demonstrated in homologous species skin-testing extracts of different laboratory sources.

REFERENCES

1. Cohen, S. G.: Immune Sera in the Study of Food Antigens. I. Fish, J. ALLERGY **30**: 250, 1959.
2. Leone, C. A.: Comparative Serology of Some Brachyuran Crustaceae and Studies in Hemocyanin Correspondence, Biol. Bull. **97**: 273, 1949.
3. Boyden, A.: The Measurement and Significance of Serological Correspondence Among Proteins. In Cole, W. H. (editor): Serological Approaches to Studies of Protein Structure and Metabolism, New Brunswick, 1954, Rutgers University Press, pp. 74-97.
4. Tuft, L., Blumstein, G. I., and Usher, S.: Studies in Food Allergy. I. Antigenic Relationship of Shellfish, J. ALLERGY **11**: 475, 1940.
5. Perlman, F.: Insects as Inhalant Allergens; Consideration of Aerobiology, Biochemistry, Preparation of Material, and Clinical Observations, J. ALLERGY **29**: 302, 1958.
6. Way, K. D.: Water Flea Sensitivity; Case Report, J. ALLERGY **12**: 495, 1940.
7. Charpin, I.: Semaine d. hôp. Paris, Oct. 26, 1957, cited in J. A. M. A. **163**: 762, 1957.
8. Perlman, F.: Personal Communication.
9. Sherman, W. B., and Kessler, W. R.: Allergy in Pediatric Practice, St. Louis, 1957, The C. V. Mosby Company, p. 104.

The Mechanism of the Schultz-Dale Reaction

Walton B. Geiger, Ph.D., and Hilma S. Alpers, M.A., San Antonio, Texas

INTRODUCTION

SMOOTH muscle from an antigenically sensitized animal contracts upon re-exposure to antigen. This response, discovered by Schultz[1] and studied extensively by Dale,[2] has come to be considered the first step in many local and general anaphylactic reactions.[3] The similarity between the Schultz-Dale reaction and the contraction due to cholinergic stimulation has long been recognized. With guinea pig ileum, for example, both responses are potentiated by eserine and diminished by atropine.[5] Also, liberation of an acetylcholine-like stimulant during the Schultz-Dale reaction has been reported.[6] More direct evidence for the involvement of nerve in the Schultz-Dale reaction has recently been obtained by showing that the reaction is prevented by the presence of small concentrations of narcotics, such as alcohols, urethanes, or local anesthetics.[7] Certain isolated bits of published information foreshadowed these observations.[8-10] More detailed study has shown that the higher alcohols and urethanes act at more peripheral sites than the lower ones (Fig. 1). As the Schultz-Dale reaction remains unaffected in the presence of ganglionic blocking agents, such as tetraethylammonium and hexamethonium, the sites of action of antigen seem to be postganglionic.

Because it was found in some of our earlier experiments that stimulation by both antigen and serotonin was prevented by structural analogues of serotonin, it was reasoned that the antigen-antibody reaction might cause liberation of serotonin. However, since it was also observed that antigen stimulation was blocked by botulinum toxin while serotonin stimulation was not, it was suggested that liberation of serotonin by antigen might be the process blocked by botulinum toxin.

In the literature on the Schultz-Dale reaction and on anaphylactic reactions generally,[3, 12, 13] much attention is given to the possible involvement of histamine and acetylcholine. The present article offers evidence for a hypothesis that includes functions for these substances as well as for serotonin.

EXPERIMENTAL

Guinea pigs, each weighing about 500 grams, were sensitized to crystalline egg albumin by 2.0 mg. intraperitoneal injections on two successive days. After

From Trinity University and the Southwest Foundation for Research and Education.
Supported by Grant G-2500 from the National Science Foundation.
Received for publication Jan. 5, 1959.

twenty-one to twenty-four days the animals were killed. The ileum was removed and washed with magnesium-free Tyrode's solution.[14] Portions of the ileum about 15 mm. long were set up in a muscle bath stirred by aeration and arranged for washing by overflow. It was found important to keep the lumen open at both ends. The bath and all solutions were kept at 37.5° C. Contractions were recorded with an ink-writing lever that exerted a tension of about 0.5 gram and gave an amplification of 1:4.5. The usual method of testing the effect of a substance of interest upon the action of antigen and other stimulants was to add the substance to the bath containing the ileum and, after fifteen minutes, to add antigen or another stimulant of interest. Ordinarily, each stimulant was applied only once to a particular portion of ileum. All experimental results reported were confirmed with several animals. Every third experiment was normally a test with a "control" strip not exposed to any supposed inhibitor. Particular care was taken to avoid artifacts due to the history of the strips. For example, it had been noted that previous exposure to histamine could diminish somewhat the sensitivity to antigen and that previous exposure to a little serotonin often increased sensitivity to antigen. For such reasons, nearly all tests were made with strips of ileum not previously exposed to other stimulants. It was found important to remove a stimulating contaminant, apparently ammonium sulfate, from the egg albumin before use.

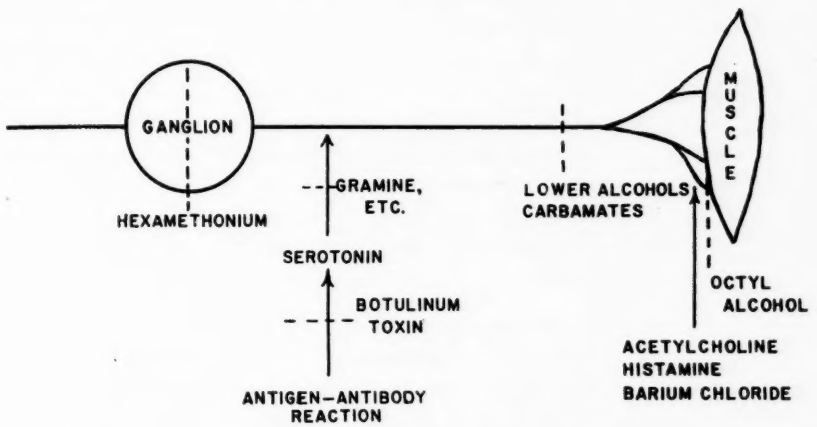


Fig. 1.—A diagrammatic representation of hypothetic sites of action of antigen, of other stimulants, and of inhibitors on guinea pig ileum.

This was done by dialyzing the egg albumin solutions against Tyrode's solution. As the amount of egg albumin necessary for a satisfactory response varied from one animal to another, the level to be used was chosen by first determining the minimum concentration of egg albumin needed for a just perceptible response and then using ten times this concentration in the tests with this particular animal.

Experiments in which the heart of *Venus mercenaria* was used to detect acetylcholine liberation were based on the techniques of Welsh and Taub.[16] Samples of ileum, each weighing approximately 2 to 3 grams, from guinea pigs

sensitized to egg albumin were immersed in 6.0 ml. of a balanced salt solution* containing 0.03 mg. eserine per milliliter at 37.5° C. Egg albumin solution, usually 0.3 mg. per milliliter, was added. After one minute the solution was poured off, and an equal volume of a mixed salt solution of appropriate concentration was added, so that the final composition was that of sea water.[16] This solution was then chilled to 15° C. and assayed for acetylcholine, as described by Welsh and Taub.

RESULTS

1. *Histamine.*—The probable involvement of histamine in allergic reactions has been recognized for many years. The evidence for this is of two kinds. First of all, liberation of histamine from tissues of sensitized animals by antigen has been demonstrated many times. Although the literature is too voluminous for complete citation here, one may mention the reviews of Feldberg[17] and of Code,[18] and the historically important papers of Gebauer-Fuellneg and associates,[19] and of Bartosch and colleagues.[20] Histamine liberation is supported by both pharmacologic and chemical data, but, as much of this work preceded the discovery of serotonin, it must be accepted with some reservation.

The second kind of evidence is based on the suppression of the Schultz-Dale reaction by antihistaminics.[21, 22] This evidence likewise is not completely satisfying, as these drugs are incompletely specific and able to prevent stimulation of smooth muscle by acetylcholine and histamine, but at concentrations higher than those required to inhibit stimulation due to histamine.[23] The concentrations of antihistaminics required to suppress the Schultz-Dale reaction have been shown by Alberty and Schiede[24] to be about four times as large as those effective against histamine. With our animals, tripelennamine hydrochloride, 2.0 mcg. per milliliter, completely suppressed the action of histamine hydrochloride, 0.05 mcg. per milliliter, usually without appreciably changing the response to antigen.

Strong evidence for the involvement of a second humoral agent has been provided by Schild's[25] well-known experiment. Schild desensitized guinea pig uterus to histamine by exposing it to a large concentration of this substance and found that uterus so treated still responded to antigen. Thus, although a histamine function in the Schultz-Dale reaction seems to have strong experimental support, this substance seems unlikely to be the sole agent involved. Evidence for the simultaneous formation of histamine and serotonin is presented in the next section.

2. *Serotonin.*—Our attention was first drawn to the possibility that serotonin might be involved in the Schultz-Dale reaction of guinea pig ileum by the observation that the response was prevented in the presence of certain serotonin antagonists: gramine, bufotenin, and yohimbine. More recently Fink and Gardner[26, 27] have shown that these substances also have antihistaminic action.

*Artificial sea water,[16] diluted 1:4, was used. This solution has a composition near that of Tyrode's solution.

TABLE I. EFFECT OF STRUCTURAL ANALOGUES OF SEROTONIN ON STIMULATION BY SEROTONIN AND HISTAMINE

SEROTONIN ANALOGUE	CONCENTRATION OF ANALOGUE JUST INHIBITORY TO: SEROTONIN CREATININE SULFATE 2.0 MCG./ML. (MCG./ML.)	HISTAMINE DIHYDROCHLORIDE 0.2 MCG./ML. (MCG./ML.)
Gramine	30	50
Yohimbine	50	100
Bufotenin	75	150

It will be seen that with our strain of guinea pigs (Table I) these substances have both an antiserotonin and an antihistaminic action, the latter requiring about twice as great an inhibitor concentration as the former. By selecting appropriate concentrations of one of these serotonin analogues (yohimbine) and of an antihistaminic (tripelennamine), it was possible to show

TABLE II. EFFECTS OF DESENSITIZING CONCENTRATIONS OF SEROTONIN

	REACTION, IN THE PRESENCE OF INDICATED SUBSTANCES, TO: SEROTONIN CREATININE SULFATE 2.0 MCG./ML.	HISTAMINE DIHYDROCHLORIDE 0.2 MCG./ML.	ANTIGEN
Serotonin 50.0 mcg./ml.	−	+	+
Tripelennamine 2.0 mcg./ml.	+	−	+
Serotonin 50.0 mcg./ml. Tripelennamine 2.0 mcg./ml.	−	−	−

that under conditions in which these substances singly caused little interference with the response to antigen (Fig. 2, tracings *A* and *B*) the substances together suppressed the response completely (tracing *C*). These results, summarized in Table II, suggested that both serotonin and histamine have a function in the Schultz-Dale reaction.

TABLE III. EFFECT OF YOHIMBINE AND TRIPELENNAMINE

	REACTION, IN THE PRESENCE OF INDICATED SUBSTANCES, TO: SEROTONIN CREATININE SULFATE 2.0 MCG./ML.	HISTAMINE DIHYDROCHLORIDE 0.2 MCG./ML.	ANTIGEN
Yohimbine 50 mcg./ml.	−	+	+
Tripelennamine 2.0 mcg./ml.	+	−	+
Yohimbine 50 mcg./ml. Tripelennamine 2.0 mcg./ml.	−	−	−

Schild's experiment, mentioned in the introduction, led us to perform somewhat analogous experiments with serotonin. Accordingly, ileum was exposed to serotonin in sufficient concentrations to render it insensitive to further additions of this substance[28] and was then treated with antigen and with histamine.

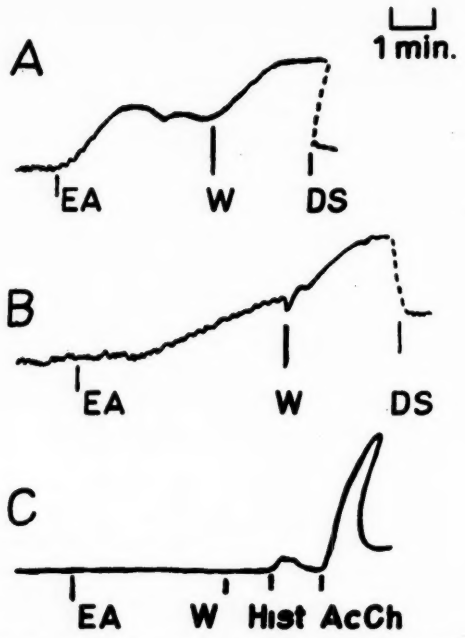


Fig. 2.—The effect of yohimbine and tripelennamine upon the Schultz-Dale reaction. *A*, In the presence of yohimbine, 50 mcg. per milliliter; *B*, in the presence of tripelennamine hydrochloride, 2.0 mcg. per milliliter; *C*, in the presence of both yohimbine and tripelennamine. *EA*. Egg albumin added; *W*, tissue washed with Tyrode's solution; *DS*, rotation of recording drum stopped for fifteen minutes; *Hist*, histamine dihydrochloride (0.05 mcg. per milliliter) added; *AcCh*, acetylcholine bromide (0.02 mcg. per milliliter) added.

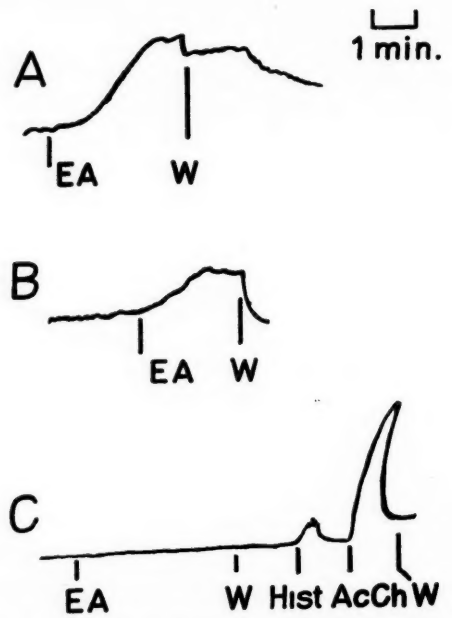


Fig. 3.—The effect of preliminary desensitization to serotonin upon the Schultz-Dale reaction, in the absence or presence of tripelennamine. *A*, Effect of antigen on serotonin-treated ileum; *B*, effect of antigen on ileum (not exposed to serotonin) in the presence of tripelennamine; *C*, effect of antigen upon serotonin-treated ileum in the presence of tripelennamine. (Abbreviations and concentrations are the same as in Fig. 2.)

Both substances caused contraction (Table III). This result indicated the involvement of a second humoral agent in addition to serotonin, probably histamine. The experiment was therefore repeated, with the modification that an antihistaminic (tripelennamine), was added at a concentration that was sufficient to block histamine but not to affect the response of untreated ileum to serotonin or antigen. This combination of treatments completely prevented contraction due to antigen. The pertinent kymograph tracings are presented in Fig. 3, in which tracing *A* shows the response to antigen after desensitization to serotonin, tracing *B* shows the response in the presence of tripelennamine, and tracing *C* shows the lack of response of serotonin-desensitized ileum in the presence of tripelennamine to antigen. The recent observations and conclusions by Gaddum and Picarelli[29] have provided a basis for further clarification. These investigators have suggested that there are two kinds of serotonin receptors in ileum—one kind (M receptor) associated with nerve and blocked by morphine and atropine and another kind (D receptor) associated with muscle and blocked by lysergic diethylamide (LSD), bromolysergic diethylamine (BOL), and Dibenzyline. Thus, it would be expected that, to suppress effectively the Schultz-Dale reaction three kinds of receptors must be blocked: the M receptors, the D receptors, and the histamine receptors.

TABLE IV. EFFECTS OF COMBINATIONS OF INHIBITORS

INHIBITORS	REACTION, IN THE PRESENCE OF INHIBITOR, TO:		
	SEROTONIN 1.0 MCG./ML.	HISTAMINE 0.05 MCG./ML.	ANTIGEN
LSD-25	+	+	+
Atropine	+	+	+
Tripelennamine	+	−	+
LSD-25 Tripelennamine	+	−	+
LSD-25 Atropine	−	+	+
Atropine Tripelennamine	+	−	S*
LSD-25 Atropine Tripelennamine	−	−	−

Concentrations:
LSD-25, 0.5 meg./ml.
Atropine sulfate, 20 meg./ml.
Tripelennamine hydrochloride, 2.0 meg./ml.

*S = Reaction largely suppressed.

An experimental test of this possibility was made by using atropine or morphine to block the M receptors, LSD to block the D receptors, and tripelennamine to block the histamine receptors (Table IV). Used singly, atropine, morphine, LSD, and tripelennamine did not prevent the Schultz-Dale reaction (Fig. 4). A study of the effects of combinations of these agents showed that

the dual combinations LSD plus atropine (Fig. 5, tracing *A*), LSD plus tripelennamine (tracing *B*), or atropine plus tripelennamine (tracing *C*) did not prevent the response to antigen, although the final pair often considerably reduced its magnitude. The combination of pyribenzamine plus atropine was also ineffective. The triple combination of LSD, pyribenzamine, and atropine (tracing *D*) was regularly more effective, although, as will be seen, it did not abolish completely the response to antigen but very markedly diminished its amplitude and speed. This result suggests either the involvement of an additional unidentified stimulant, such as the "slow-reacting substance" of Trevethie, or a lack of perfection in the effectiveness and specificity of the inhibitors.

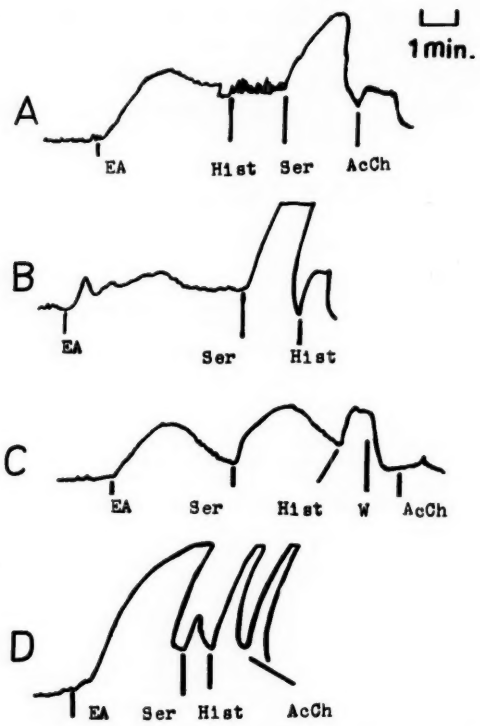


Fig. 4.—The effect of certain substances, singly, upon the Schultz-Dale reaction. *A*, Tripelennamine hydrochloride, 2.0 mcg. per milliliter; *B*, lysergic diethylamide, 0.5 mcg. per milliliter; *C*, atropine sulfate, 20 mcg. per milliliter; *D*, morphine hydrochloride, 1.0 mcg. per milliliter. Serotonin concentration was 1.0 mcg. per milliliter; other concentrations and abbreviations are the same as in the previous figures.

An analogous study was made in which morphine was used as inhibitor for the M receptor (Table V). In this instance, one of the dual combinations, LSD and morphine (Fig. 6, tracing *A*), did not prevent the response to antigen, although another, pyribenzamine and morphine (tracing *B*), was practically as effective as the triple combination (tracing *C*).

Experiments were also performed in which Dibenzyline or one of the similar compounds (Dibenamine or Abbott SY-47) was used. Results were

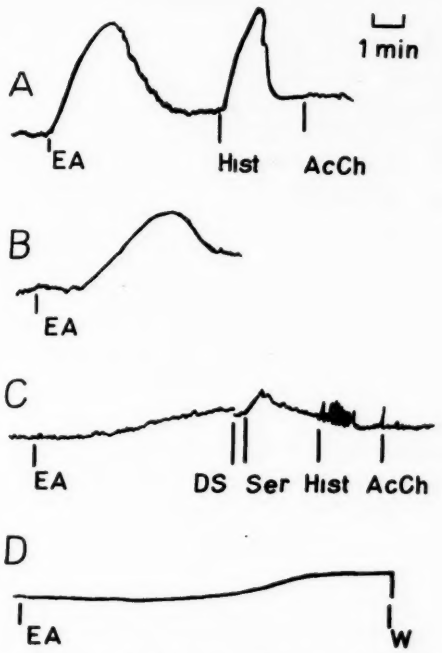


Fig. 5.—Effect of combinations of inhibitors upon the Schultz-Dale reaction. *A*, Atropine and lysergic diethylamide; *B*, tripelennamine and lysergic diethylamide; *C*, atropine and tripelennamine; *D*, atropine, lysergic diethylamide, and tripelennamine. (Abbreviations and concentrations are as previously given.)

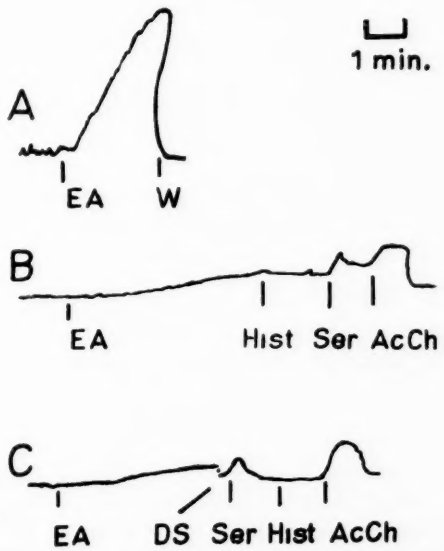


Fig. 6.—Effect of combinations of inhibitors upon the Schultz-Dale reaction. *A*, Lysergic diethylamide and tripelennamine; *B*, morphine and tripelennamine; *C*, morphine, lysergic diethylamide, and tripelennamine. (Abbreviations and concentrations are as previously given.)

highly variable. In some groups of animals, interference with the Schultz-Dale reaction was observed with Dibenzyline alone and in others the substance was less effective. Since Dibenzyline has both an antihistaminic action and an anti-serotonin (D receptor) action, this variability is not too surprising and would seem to be due to variations from animal to animal and from strip to strip in the amounts of serotonin and histamine present and in the frequency of occurrence of the different receptors.

3. *Acetylcholine.*—A number of investigators, especially Danielopolu and colleagues[5] and Nakamura,[6] have suggested the participation of acetylcholine in the Schultz-Dale reaction. Their conclusions have been based on such evidence as the suppression of the reaction by atropine, its potentiation by eserine, and the liberation of a cholinergic substance from tissues into the surrounding medium upon exposure to antigen.

TABLE V. EFFECTS OF COMBINATIONS OF INHIBITORS

INHIBITORS	REACTION, IN THE PRESENCE OF INHIBITOR, TO: SEROTONIN 1.0 MCG./ML.	HISTAMINE 0.05 MCG./ML.	ANTIGEN
LSD-25	+	+	+
Morphine	+	+	+
Tripelennamine	+	–	+
LSD-25 Morphine	S*	+	+
LSD-25 Tripelennamine	+	–	+
Tripelennamine Morphine	–	–	–
LSD-25 Tripelennamine Morphine	–	–	–

Concentrations:
LSD-25, 0.5 mcg./ml.
Morphine hydrochloride, 1.0 mcg./ml.
Tripelennamine hydrochloride, 2.0 mcg./ml.

*S = Reaction largely suppressed.

We have attempted also to demonstrate liberation of acetylcholine from sensitized tissues by antigen, by using as a test object the sensitive and specific heart of *Venus mercenaria.* Because guinea pig ileum forms small amounts of acetylcholine, even when at rest,[30, 31] the experiments were directed toward measuring any increase after the addition of antigen. Results of a typical experiment are presented in Fig. 7. Here it is seen that a strip of unstimulated ileum formed upon treatment with antigen, 0.02 mcg. of acetylcholine in the first minute per gram of tissue (tracing *A*), and an adjacent strip without antigen liberated 0.01 mcg. (tracing *B*) of acetylcholine per minute (tracing *B*). The effect of acetylcholine, at a 0.03 mcg. level, on this same heart is shown in tracing *C*. Additional evidence suggests that the product is actually acetylcholine; the active substance is destroyed by horse serum and by dilute alkali

and had no action on hearts that had previously been treated with benzoquinonium. It may be mentioned also that *Venus* heart is insensitive to histamine and does not respond to serotonin after exposure to benzoquinonium.

As the increase in acetylcholine liberation is moderate, it seems highly probable that this substance is merely pressed out of the tissue by the contraction or is perhaps a result of stimulation of the ganglia by the contraction. However, although it is quite possible that acetylcholine liberation may be more a result than a cause of the muscle contraction of the Schultz-Dale reaction, acetylcholine may nevertheless serve to reinforce and prolong this contraction.

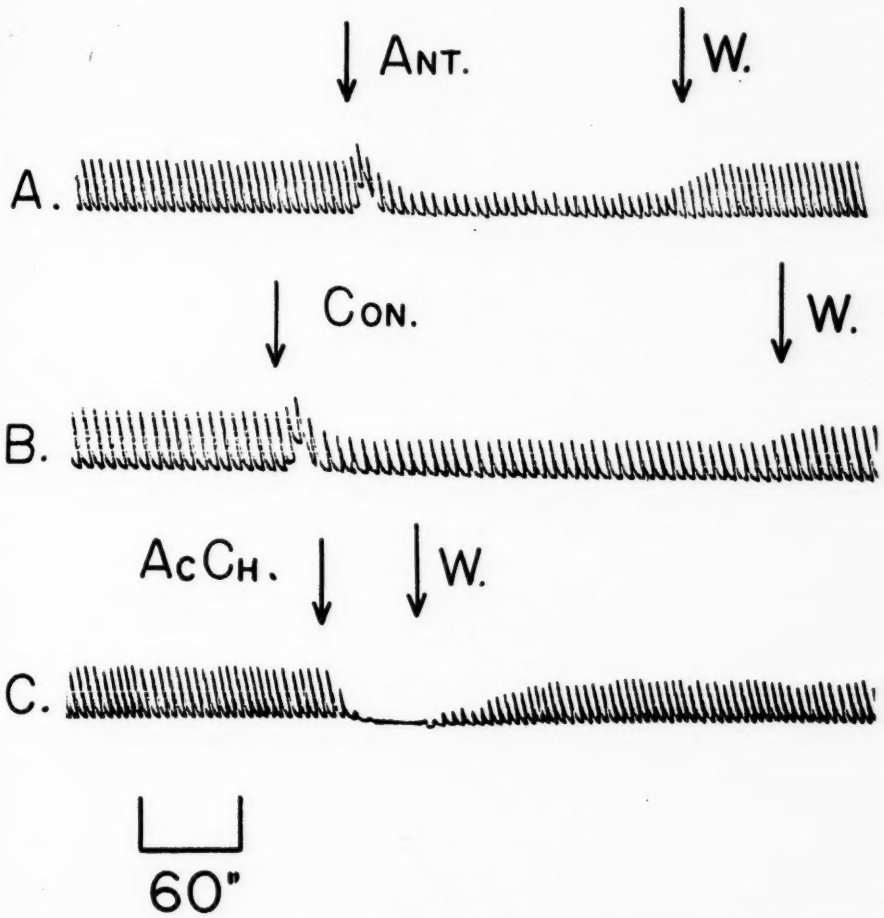


Fig. 7.—Effect upon *Venus* heart of a substance liberated from ileum as a result of stimulation by antigen (*A*) and without stimulation (*B*). Tracing *C* illustrates the sensitivity of this same heart to acetylcholine.

DISCUSSION

Present evidence concerning the mechanism of the Schultz-Dale reaction can be summarized diagrammatically (Fig. 8). Pathways of stimulation are indicated by solid arrows, and hypothetic points of action of inhibitors are

shown by broken lines. It appears probable that an antigen-antibody reaction liberates histamine and serotonin from a cellular source and that the histamine and serotonin then excite their usual receptors, either in nerve or in muscle. The diagram given applies best to guinea pig ileum. Mouse uterus, which seems to lack both histamine receptors and serotonin receptors, would be represented by a portion of the diagram. The blood vessels of the rabbit ear, which lack serotonin receptors but include histamine receptors,[32] would be represented by a somewhat different portion of the diagram.

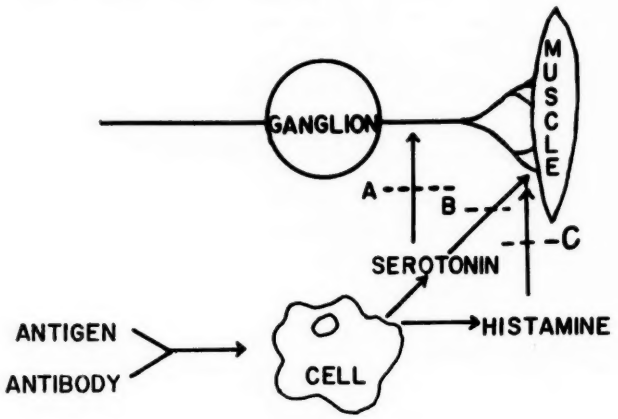


Fig. 8.—A diagrammatic representation of hypothetic pathways of action of antigen, serotonin, and histamine.

The literature contains much evidence that both serotonin and histamine are formed and stored intracellularly. This function seems well demonstrated for mast cells.[33, 34] Platelets may also store these substances.[35] Release of histamine and serotonin from whole blood and from blood platelets on addition of antigen has been demonstrated.[36] Release from mast cells under similar conditions seems, though reasonable, not yet to have been demonstrated directly, although other agents produce such release.[37, 38] The fact that mast cells are often located along nerve fibers[39] places them in a location suitable for the performance of a function in the Schultz-Dale reaction.

Thus, as a working hypothesis, it may be suggested that an antigen-antibody reaction, perhaps by activating an enzyme, causes release of serotonin and histamine from a cellular or other formed source. The serotonin and histamine then seem to stimulate muscle by acting upon the appropriate receptors in nerve or in muscle. In terms of this hypothesis, probable sites of action of the principal inhibitors of the Schultz-Dale reaction would seem to be as follows:

1. Botulinum toxin—Prevents release of serotonin and histamine.
2. Lower alcohols, urethanes—Prevent conduction of nerve impulse to muscle.
3. Higher alcohols—Block a more peripheral conducting system.
4. LSD—Blocks Gaddum's D receptors.
5. Morphine, atropine—Blocks Gaddum's M receptors.

6. Yohimbine, gramine, bufotenin—Block M receptors, D receptors, and, at higher concentrations, histamine receptors.
7. Dibenzyline—Blocks D receptors and histamine receptors.

SUMMARY

1. Evidence is presented supporting involvement of at least three humoral agents—histamine, serotonin, and acetylcholine—in the contraction of ileum from sensitized guinea pigs upon re-exposure to antigen in vitro (the Schultz-Dale reaction).

2. The Schultz-Dale reaction is suppressed completely only in the presence of a combination of agents capable of blocking both the histamine receptors and the two kinds of serotonin receptors present in the tissue.

3. This complete suppression of the reaction may be accomplished in three ways: (1) by blocking the three kinds of receptor separately, as by adding simultaneously an antihistaminic, atropine, and LSD; (2) by first exposing the tissue to a large concentration of serotonin, thereby desensitizing it to this substance, and then adding an antihistaminic; and (3) by the simultaneous use of one of the less specific antiserotonin agents (yohimbine) and an antihistaminic.

4. The liberation of acetylcholine during the Schultz-Dale reaction has been confirmed but seems to be a result, rather than a cause, of the contraction.

The research described in the section headed "Acetylcholine" was largely performed at Irvington House, Irvington-on-Hudson, New York, with the assistance of Miss Elizabeth M. Hill and Mrs. Mabelle Thompson and with support of a grant from the Helen Hay Whitney Foundation.

REFERENCES

1. Schultz, W. H.: Physiological Studies in Anaphylaxis. I. The Reaction of Smooth Muscle of the Guinea Pig Sensitized With Horse Serum, J. Pharmacol. & Exper. Therap. **2:** 221, 1910.
2. Dale, H. H.: The Anaphylactic Reaction of Plain Muscle in the Guinea Pig, J. Pharmacol. & Exper. Therap. **4:** 167, 1913.
3. Seegal, B.: Experimental Anaphylaxis in Lower Animals, Ann. New York Acad. Sc. **50:** 681, 1949.
4. Auer, J.: The Prophylactic Action of Atropine in Immediate Anaphylaxis of Guinea Pigs, Am. J. Physiol. **26:** 439, 1910.
5. Danielopolu, D., Rudeanu, A., Bruckner, I., and Simionescu, S.: Libération d'acétylcholine dans le choc paraphylactique, Compt. rend. Soc. biol. **142:** 757, 1948.
6. Nakamura, K.: Nouvelle opinion sur la production du choc anaphyactique, spécialement sur le rôle de l'acétylcholine, dans l'anaphylaxie, Jap. J. Exper. Med. **19:** 31, 1941.
7. Geiger, W. B., Hill, E. M., and Thompson, M.: A Study of the Mechanism of the Schultz-Dale Reaction, Proc. Soc. Exper. Biol. & Med. **92:** 793, 1956.
8. Guillot, M., and Ong, S. G.: Influence de diverses substances capillaroactives sur la contraction anaphylactique *in vitro* de l'uterus du cobaye sensibilisé, Compt. rend. Soc. Biol. **126:** 314, 1937.
9. Gray, W. D., Pedrick, L., and Winne, R.: Effect of Cortisone on the Anaphylactic Response of Guinea-Pig Ileum, Proc. Soc. Exper. Biol. & Med. **78:** 679, 1951.
10. Mongar, J. L., and Schild, H. O.: Inhibition of the Anaphylactic Reaction, J. Physiol. **135:** 301, 1957.
11. Geiger, W. B., and Alpers, H. S.: Mode of Action of Antigen and Other Smooth-Muscle Stimulants, Science **125:** 1141, 1957.
12. Dragstedt, C. A.: Anaphylaxis, Physiol. Rev. **21:** 563, 1932.
13. Dale, H. H.: The Biological Significance of Anaphylaxis, Proc. Roy. Soc. (London), s.B **91:** 126, 1920.

14. Campbell, D. H., and McCasland, G. E.: *In Vitro* Anaphylactic Response to Polyhaptenic and Monohaptenic Simple Antigens, J. Immunol. **49:** 315, 1944.
15. Tower, D. B., and McEachern, D.: Experiences With the Venus Heart Method for Determining Acetylcholine, Canad. J. Research, Sect. E **26:** 183, 1948.
16. Welsh, J. H., and Taub, R.: The Action of Choline and Related Compounds on the Heart of *Venus mercenaria*, Biol. Bull. **95:** 346, 1948.
17. Feldberg, W.: Histamine and Anaphylaxis, Ann. Rev. Physiol. **3:** 671, 1941.
18. Code, C. F.: Histamine in Blood, Physiol. Rev. **32:** 47, 1952.
19. Gebauer-Fuellneg, E., Dragstedt, C. A., and Mullenix, R. B.: Observations on a Physiologically Active Substance Appearing During Anaphylactic Shock, Proc. Soc. Exper. Biol. & Med. **29:** 1084, 1932.
20. Bartosch, R., Feldberg, W., and Nagel, E.: Das Freiwerden, eines histaminähnlischen Stoffes bei der Anaphylaxie des Meerschweinchens, Pflüger's Arch. ges. Physiol. **230:** 129, 1932.
21. Rapport, M., and Koelle, G. B.: The Action of Antihistaminics and Atropine in Blocking the Spasmogenic Activity of Serotonin on the Guinea-Pig Ileum, Arch. Internat. Pharmacodyn. **92:** 464, 1953.
22. Engelhardt, G., and Hahn, G.: Pharmakologische Beinflussung anaphylaktischer Reaktionen an Gefässpräparaten, Arch. exper. Path. u. Pharmakol. **231:** 507, 1957.
23. Loew, E. R.: Pharmacology of Antihistamine Compounds, Physiol. Rev. **27:** 542, 1947.
24. Alberty, J., and Schiede, M.: Untersuchungen über die anaphylactische Reaktion des isoierten Meerschweinchendarmes, Arch. Internat. Pharmacodyn. **96:** 28, 1953.
25. Schild, H. O.: The Reaction of the Guinea-Pig's Uterus Immersed in a Histamine Solution to Histamine and Anaphylaxis, J. Physiol. **86:** 51 *P*, 1936.
26. Fink, M. A.: Anaphylaxis in the Mouse: Possible Relation of the Schultz-Dale Reaction to Serotonin Release, Proc. Soc. Exper. Biol. & Med. **92:** 673, 1956.
27. Fink, M. A., and Gardner, C. E.: Anaphylaxis in the Guinea Pig: Improbability of Release of Serotonin in the Schultz-Dale Reaction, Proc. Soc. Exper. Biol. & Med. **97:** 554, 1958.
28. Gaddum, J. H., and Hameed, K. H.: Drugs Which Antagonize 5-Hydroxytryptamine, Brit. J. Pharmacol. **9:** 240, 1954.
29. Gaddum, J. H., and Picarelli, Z. P.: Two Kinds of Serotonin Receptor, Brit. J. Pharmacol. **12:** 323, 1957.
30. Feldberg, W. S.: On the Origin and Function of Acetylcholine in the Intestinal Wall, Proc. Roy Soc. (London), s.B **137:** 285, 1950.
31. Feldberg, W.: The Action of Histamine on the Blood Vessels of the Rabbit, J. Physiol. **63:** 211, 1937.
32. Riley, J. F., and West, G. B.: The Presence of Histamine in Tissue Mast Cells, J. Physiol. **120:** 528, 1953.
33. Benditt, E. P.: 5-Hydroxytryptamine in Mast Cells, Proc. Soc. Exper. Biol. & Med. **90:** 303, 1955.
34. Humphrey, J. H., and Jacques, R.: The Histamine and Serotonin Content of the Platelets and Polymorphonuclear Leucocytes of Various Species, J. Physiol. **124:** 305, 1954.
35. Humphrey, J. H., and Jacques, R.: Liberation of Histamine Platelets by Antigen-Antibody Reactions *In Vitro*, J. Physiol. **119:** 43 *P*, 1953.
36. Benditt, E. P., and Rowley, D.: 5-Hydroxytryptamine and Histamine as Mediators of the Vascular Injury Produced by Agents Which Damage Mast Cells in Rats, J. Exper. Med. **103:** 399, 1956.
37. Parratt, J. R., and West, G. B.: Release of 5-Hydroxytryptamine and Histamine From Tissues of the Rat, J. Physiol. **137:** 179, 1957.
38. Padawer, J.: Studies on Mammalian Mast Cells, Tr. New York Acad. Sc. **19:** 690, 1957.

On Modification of Penicillin Antigenicity

George H. Berryman, M.D., and Elmer W. Fisherman, M.D., Chicago, Ill.

SENSITIVITY reactions to penicillin are estimated to have an incidence of at least 5 per cent and constitute a calculated risk when this antibiotic is employed therapeutically. Nevertheless, penicillin is still used frequently because of its excellent bactericidal properties in diseases of streptococcal, pneumococcal, gonococcal, and spirochetal origin. Particularly in children, where the frequency of sensitivity reactions is reportedly lower than in the adult, penicillin continues to have wide use, both therapeutically and prophylactically.

With continuing exposure of the public to penicillin, however, the question of increasing sensitization deserves thought. Not only is the patient likely to receive this antibiotic when ill; he is also likely to be exposed to small but immunologically significant amounts of this agent incidentally—in milk,[1] vaccines, troches, nasal sprays, etc.[2]

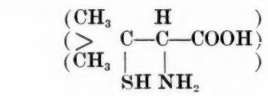
The incidence of serious penicillin reactions being encountered when penicillin is employed therapeutically in a population so exposed has been described by Welch,[3] who determined from a survey of 800 hospitals that 80 per cent of the 2,905 antibiotic reactions occurring in those institutions from 1953 to 1957 were associated with the use of penicillin. The disturbing statistics revealed that (1) 900 life-threatening reactions involved penicillin, two-thirds of these following intramuscular administration, and (2) of these, there were eighty-three deaths resulting from anaphylactic shock, all but one following intramuscular use of penicillin. While this incidence of reactions is comparatively low in proportion to the number of persons who must have received either oral or parenteral penicillin during that period, it must be appreciated that in the general population a significant number of sensitivity reactions go unrecognized, untreated, or unreported.

The various types of penicillin reactions, as well as precautions and treatments, have been the subject of excellent articles,[4] and the clinical aspects have been ably presented by Feinberg and associates.[5, 6] Samter and Berryman[7] have recently summarized some of the theoretical aspects of drug sensitivity and suspect that a cellular deficiency in the host of enzymatic, conjugating, or detoxifying mechanisms may be a prerequisite for the development of drug sensitivities. Whatever the role of the host, however, it is the

From the Allergy Unit, University of Illinois.
Received for publication Jan. 5, 1959.

chemical or physicochemical structure of the drug that determines its antigenicity. We may ask what chemical, physical, or spatial characteristics enable the penicillin molecule to act in certain persons as a complete antigen or as a hapten.

We have investigated the cross-reactivity among penicillin G and several of its derivatives in passively sensitized skin areas of nonallergic recipients. In local sites injected with the serum of patients known to contain reagins to penicillin G, we have tested the following substances: (1) penicillin G; (2) penicillin V; (3) thio-penicillin; (4) thenyl penicillin; (5) synnematin (the only penicillin in this series without a ring structure in the side chain); and (6) penicillamine (a degradation product derived from the penicillin molecule and having the chemical identity of dimethylcysteine).



The structural relationships of the various penicillins are shown in Fig. 1, in which the portion of the molecule to the right of the dotted line is common to all, but the portion to the left of the dotted line differs (that is, only the side chain varies).

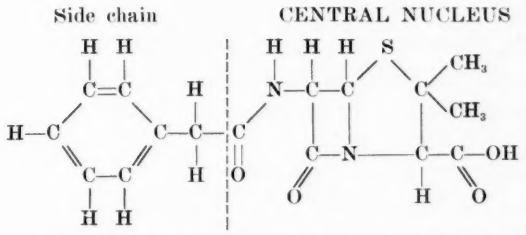
Upon hydrolysis, chemically different moieties are obtained from the side chains: penicillin G → phenyl acetic acid; penicillin V → phenoxy acetic acid; thenyl penicillin → thenyl acetic acid; thio-penicillin → phenyl mercapto acetic acid; synnematin → alpha amino adipic acid, a straight-chain aliphatic structure, contrasting to the ring structures common to all the preceding compounds.

Standard passive transfer techniques were used. We selected a serum with a high reagin content* obtained from a patient who had been hospitalized for urticaria and angioedema due to penicillin G. The serum was obtained under sterile precautions from blood shown to be Kahn-negative. One-tenth milliliter of this penicillin-sensitive serum was introduced intracutaneously into volar sites of recipient patients who had no history of having received therapeutic penicillin at any time in the past. The recipients were ambulant patients who suffered from various diseases encountered in a general hospital; none was seriously ill, and none had allergic disease.

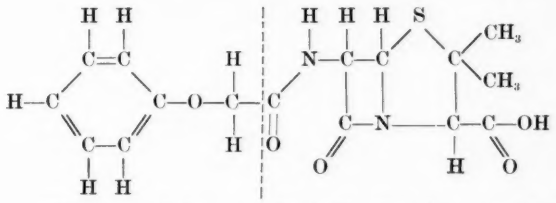
Two days after the preparatory injection, 0.01 to 0.02 ml. injections of the sterile test antigen solutions were introduced, one by one, into the passively sensitized skin sites. The concentration of all test materials was standardized to the equivalent of 10,000 U. per milliliter.† The appearance and development of the localized reactions were timed and measured by ruler.

*Not all sera from patients recovering from a penicillin reaction are suitable for passive transfer testing. Thus, of the sera obtained from ten such cases, only two contained appreciable titers of circulating antibodies. The serum utilized for the present study gave a positive passive transfer test to as small a quantity as 4 to 8 units of penicillin G.

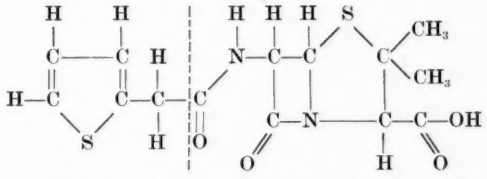
†The synnematin employed had a 75.8 per cent purity; the 24.2 per cent impurities are probably inactive synnematin or derivatives therefrom.



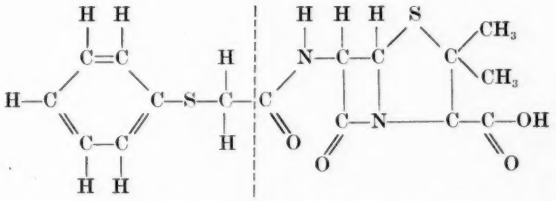
(Benzyl group) Penicillin G (benzyl penicillin)



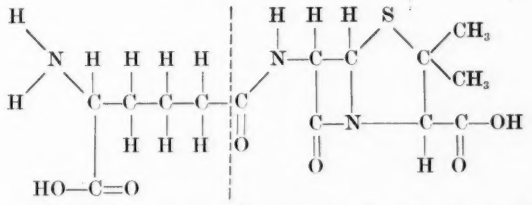
(Phenoxymethyl group) Penicillin V (phenoxymethyl penicillin)



(Thenyl group) Thenyl penicillin



(Phenylmercapto methyl group) Penicillin S (thio-penicillin)



(4-amino 4 carboxy n-butyl group) Synnematin B or cephalosporin N

Fig. 1.—Structural formulas of penicillins.

Control (sensitized) sites were injected with saline to rule out the possibility that trauma or dermographism might contribute to positive reactions observed in the test sites. Control injections of the test solutions were also made into nonsensitized areas of the skin to eliminate the possibility that reactions were due to primary irritation.

RESULTS

Pilot Studies (Passive Transfer).—When test solutions were injected into the individually prepared sites in rapid succession, that is, at intervals of twenty to thirty seconds, it was found that penicillamine, synnematin, and saline control gave much less intense reactions than the other test substances. However, we noted that on occasion some extra *untested* (passively sensitized but unused) sites also became positive (that is, without introduction of any antigen); presumably, lymphatic or vascular transport of the most reactive antigen (usually penicillin G) was producing positive reactions in the other sites. Accordingly, our technique was revised so that a twenty-minute wait was interposed between administration of each of the test solutions, and the sequence of administration was standardized as follows: synnematin, followed by penicillamine, and then penicillin G (see following section). While the results of the pilot studies were not decisive, they did give an indication that penicillins G and V, thenyl penicillin, and thio-penicillin were cross-antigenic with penicillin G, whereas synnematin and penicillamine appeared to be less cross-antigenic (Table I).

TABLE I. REACTIONS (TWENTY-FORTY MINUTES) AT SITES PREVIOUSLY PASSIVELY SENSITIZED WITH PENICILLIN G ANTISERUM, AFTER CHALLENGE WITH INDIVIDUAL ANTIGENS AT INTERVALS OF APPROXIMATELY TWENTY SECONDS

PATIENT	PENICILLIN G	PENICILLIN V	THENYL PENICILLIN	THIO-PENICILLIN	PENICILLAMINE	SYNNEMATIN	SALINE
1	++++	++++	++++	+++	+++	+	++++
2	+++	++++	++	++	++	0	++
3	+++	+++	+++	+++	0	0	0
4	+++	+++	+++	+++	0	0	0

+ = Positive reaction (1-4).
0 = No reaction.
All patients were tested with all antigens in non-prepared sites, with negative results (recipient control).

Subsequent Standardized Studies (Passive Transfer).—Table II shows the results obtained with the sequential technique in ten different recipients. Intracutaneous injection of synnematin into site I never produced a positive reaction; nor did its introduction into site I have any effect upon the other prepared sites (line A). A negative response also occurred when penicillamine was administered at site II (line B). Upon administration of penicillin G at site III, however (line C), there was an immediate positive reaction not only at site III but also at both the previously tested sites I and II as well as the untested control site IV.

Similar results were obtained when penicillin V was employed (instead of penicillin G) at site III.

One of the most striking demonstrations of the difference in antigenicity between synnematin and penicillin G was obtained in a special series of passive transfer tests in which there were contrasted the reaction from 8 units of penicillin G with those from 500 and 1,000 units, respectively, of synnematin. The use of 0.02 c.c. of 25,000 and 50,000 units per milliliter of the latter produced no wheal or erythema in sites passively sensitized to penicillin G; however, 0.02 c.c. of a solution containing 400 units per milliliter of penicillin G (that is, 8 units) produced a wheal of 0.6 cm. and erythema of 1.8 cm. within five minutes after injection.

TABLE II. TYPICAL ALLERGIC RESPONSE AT SITES PASSIVELY SENSITIZED FORTY-EIGHT HOURS PREVIOUSLY WITH 0.1 C.C. SERUM CONTAINING A HIGH TITER OF PENICILLIN G ANTIBODIES

	SITES INJECTED AT 20-MINUTE INTERVALS WITH	TIME OF CHALLENGE INJECTION AND OBSERVATION (MINUTES)	I	II	III	IV
A	Synnematin into site I	0	Intracutaneous synnematin	No injection	No injection	No injection
	Results at all sites	20	Negative	Negative	Negative	Negative
B	Penicillamine into Site II	20	No additional injection	Intracutaneous penicillamine	No injection	No injection
	Results at all sites	40	Negative	Negative	Negative	Negative
C	Penicillin G (or V) into site III	40	No additional injection	No additional injection	Intracutaneous penicillin G	No injection
	Results at all sites	60	Positive (E—3.5 cm.)	Positive (E—2.0 cm.) (W—0.5 cm.)	Marked positive (E—4.5 cm.) (W—1.5 cm.)	Positive (E—4.5 cm.)
	Continued observation of all sites	90	Negative	Positive (E—2.0 cm.) (W—0.4 cm.)	Moderate positive (E—1.0 cm.)	Positive (E—3.0 cm.)
		120	Negative	Weak positive	Weak positive	Weak positive

E = Erythema.
W = Wheal.
Note: Non-sensitized sites all negative to test antigens.

Direct Skin-Testing Studies.—The apparent lack of cross-antigenicity between synnematin or penicillamine and the penicillins made it possible to test direct skin reactions in another group of patients who were recovering from penicillin skin reactions. A total of ten patients with diagnoses of penicillin sensitivity were tested, but only two showed positive skin tests; this is in accord with the widely quoted estimate that only 10 per cent of patients with a penicillin reaction do demonstrate skin-sensitizing antibodies. The results in these two patients add further confirmation to the results obtained with the passive transfer studies. The history of one of these two patients follows.

A Negro man had received an injection of penicillin for treatment of gonococcal urethritis at a venereal disease clinic six weeks prior to this admission. Approximately three days later there had developed urticaria of the arms, thighs, and back, as well as angioedema of the lips and associated difficulty in swallowing. This episode of urticaria lasted one week and then underwent spontaneous remission. Ten days prior to this present admission the patient's urine had become "milky," and a private physician gave an injection (penicillin?). Five days thereafter urticarial wheals again appeared, followed by swelling of the arms, soreness of the feet, congested throat, and again difficulty in swallowing. The patient was brought to the hospital, and during interrogation he collapsed with a marked drop in blood pressure. His response to immediate emergency measures plus subsequent antiallergic therapy was satisfactory, and he recovered from all symptoms over the course of ten days.

TABLE III. SKIN REACTIONS (CM.) FROM DIRECT INTRADERMAL TESTS

	AFTER ½ HOUR	AFTER 1½ HOURS
Penicillin G	W 1.4 E 2.5	W 1.4 E 2.5
Penicillin V	W 2.2 E 2.2	W 5.0 E 5.0
Penicillamine	0	0
Synnematin	0	0
Control saline	0	0

W = Wheal.
E = Erythema.

Skin tests with penicillin had a dual purpose, therefore: (1) to confirm the suspected sensitivity and (2) to guide the choice of agent for future treatment. Initial precautionary scratch tests with penicillins G and V, penicillamine, and synnematin were negative. Intradermal tests with solutions containing 10,000 units per milliliter produced the results shown in Table III.

DISCUSSION

Immunologic Aspects.—Several points of clinical interest emerge from this study; they will be discussed in the sequence in which they were encountered. First, during preparation for the passive transfer tests, it was somewhat surprising to find a considerable number of recipients who did not fix the passively transferred penicillin antibodies. Whatever the explanation, it was found that seven out of fifteen prospective recipients showed no reaction of any kind to the various antigens administered two days following passive transfer of the highly reaginic serum. The same serum did produce successful results in the remaining eight patients under identical conditions of administration. This shows that a successful passive transfer test requires not only a highly reaginic serum plus potent antigen but also a recipient whose skin can fix antibodies and react by release of histamine when the specific antigen is introduced. Later, when direct skin testing of penicillin-sensitive patients was performed, it was of interest to confirm in eight out of ten patients the previously reported absence of skin reagins despite the recent manifestation of systemic sensitivity.

The data in Table II demonstrate the necessity (in routine passive transfer testing) for preliminary titration of the test antigen in order to avoid

"spill-over" and subsequent pickup of the excess antigen by other untested or control sites. The data also provide additional evidence (beyond the negative response itself) that synnematin and penicillamine were not cross-antigenic with penicillin G, for the sites where they were injected (I and II) still contained unneutralized penicillin G antibodies which were capable of reacting with the remotely administered penicillin G. The fact that the reactions at sites I and II were of less intensity can be explained possibly on the basis of less antigen (penicillin G) being delivered via the vascular or lymphatic pickup or on the basis of a blockade of the sites by the synnematin or penicillamine—a possibility which will be explored in future studies. Thus, the conclusion that there is no cross-antigenicity between synnematin or penicillamine and penicillin G is doubly supported. It appears that replacement of the usual cyclic structure of the penicillin side chain by a straight chain (synnematin) did prevent cross-reactivity between synnematin and the other structurally related penicillins (penicillins G and V, thenyl penicillin, and thio-penicillin), all of which contain a cyclic ring structure. Apparently, the benzyl or thenyl group in the penicillin G molecule is essential for cross-antigenicity among penicillin-related substances. The extensive change in the central structure of penicillin involved in the derivation of penicillamine both alters the antibiotic potential of the molecule and changes antigenicity.

Biologic Aspects.—That changes in chemical structure tend to change antibiotic activity has been known. Thus, synnematin is more effective than penicillin G against certain gram-negative organisms (notably salmonella and some proteus) but less effective against gram-positive organisms (notably streptococci, pneumococci, and nonresistant staphylococci).[8, 9] As would be expected, however, susceptibility to penicillinase activity is not affected by side-chain modification. This is shown by the ineffectiveness of both antibiotics against those strains of proteus which contain penicillinase.[10]

The change in the penicillin molecule effected by the replacement of the usual benzyl or thenyl group with the 4-amino 4 carboxy-n-butyl group confers interesting chemical properties upon synnematin. For example, synnematin is the only penicillin-like substance capable of forming a zwitterion between the amino and carboxyl groups of its side chain.

Differences in side-chain change the physicochemical or spatial characteristics of a molecule and thereby influence or determine its biologic potentialities. Thus, both the antigenic and the antibiotic activities of synnematin and penicillin differ, presumably because of their difference in side-chain structure. However, both lose their antibiotic activity when the lactam ring of the central portion of the molecule is acted upon by penicillinase. It appears, therefore, that the central portion of the penicillin molecule is essential for its broad antibiotic activity but that the side chain determines the type of antibiotic activity (gram-positive versus gram-negative). The side chain also appears to influence the antigenic activities of these molecules. However,

when we turn to another biologic system (hemagglutination), the two molecules are reportedly interchangeable for the agglutination of red blood cells sensitized to penicillin G.* One may hypothesize, then, that the potential of a molecule is plural, in accordance with the types of physicochemical or spatial groupings which it contains and that, as a consequence of this, the molecule may have an alternative of entering into any one of several biologic reactions according to the physiologic conditions of the moment or according to the absence or presence of other molecules which have a complementary or antagonistic effect.

SUMMARY AND CONCLUSIONS

1. With a passive transfer technique, it has been shown that serum reagins to penicillin G or V do not react with synnematin or penicillamine.

2. Direct intradermal tests in two persons sensitive to penicillin G were positive to penicillins G and V but negative to synnematin and penicillamine.

3. It is concluded that modification of the chemical structure of the penicillin molecule brought about in replacement of the cyclic benzyl or thenyl group by a straight-chain 4-amino 4 carboxy-n-butyl group results in loss of cross-antigenicity. Degradation of the penicillin molecule to produce penicillamine (dimethyl cysteic acid) also results in loss of cross-antigenicity.

4. The influence of chemical structure upon the immunologic and biologic potentialities of the penicillin molecule is discussed.

For helpful suggestions or technical help and information, we are indebted to a number of our associates, including Drs. J. C. Sylvester, J. E. Philip, Jay Schenk, and Max Samter.

REFERENCES

1. Vickers, H. R., Bagratuni, L., and Alexander, S.: Lancet **1**: 351, 1958.
2. Rosenthal, A.: Follow-up Study of Fatal Penicillin Reactions, J. A. M. A. **167**: 1118, 1958.
3. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B.: Antibiotic Med. **4**: 800, 1957.
4. Carr, E. A.: New England J. Med. **245**: 892, 1951.
5. Feinberg, S. M., Feinberg, A. R., and Moran, C. F.: J. A. M. A. **152**: 114, 1953.
6. Feinberg, S. M., and Feinberg, A. R.: Guest Editorial, J. A. M. A. **160**: 778, 1956.
7. Samter, M., and Berryman, G. H.: Proc. Internat. Congress of Allergy, Paris, 1958, Éditions Médicales Flammarion.
8. Heatley, N. G., and Florey, H. W.: Brit. J. Pharmacol. **8**: 252, 1953.
9. Sylvester, J.: Personal Communication, 1954.
10. Jackson, G. G., Rubenis, M., and Mellody, M.: In Welch, H., and Martí-Ibañez, F.: Antibiotics Annual, New York, 1956-1957, Medical Encylopedia, Inc., p. 740.
11. Ley, A. B., and others: Science **127**: 1118, 1958.
12. Ley, A. B.: Personal Communication, 1958.

*The serum employed in this study, however, has been reported to us to act differently against red blood cells sensitized against penicillin and synnematin. The former were agglutinated (as previously reported[11]), but the latter were not. However, some sera containing penicillin G antibodies apparently can precipitate both these types of sensitized red blood cells,[12] thus indicating that cross-antigenicity between penicillin G and synnematin can exist in the hemagglutinin type of system.

Development of Sensitivity to Penicillinase Following Its Use in Penicillin Reaction

Robert C. Weiss, M.D., and Seymour B. Crepea, M.D., Madison, Wis.*

PENICILLINASE, an enzyme derived from various bacterial sources, is a protein with a molecular weight of about 50,000. It acts by catalyzing the hydrolysis of the lactum ring of penicillin to penicilloic acid, a substance devoid of antibacterial activity. Initial studies[2, 3, 7, 12] have suggested its value in treating penicillin sensitivity reactions. Our experience with a small group of patients, particularly with respect to their formation of antipenicillinase antibodies, is described below.

METHOD

Patients with penicillin reactions of the serum sickness type were given 800,000 units of penicillinase intramuscularly as soon as they were called to our attention. In addition, each was tested intradermally with the following mixtures: penicillin in saline 500 units per cubic centimeter, penicillinase in concentrations of 5,000 and 10,000 units per cubic centimeter, penicillin 500 units plus penicillinase 5,000 units per cubic centimeter, and penicillin 1,000 units plus penicillinase 10,000 units per cubic centimeter. The mixtures were allowed to interact for thirty minutes at room temperature prior to the test, and all testing material was less than twenty-four hours old. Where possible, these tests were performed before penicillinase administration and again two to three weeks later. Ten nonsensitive persons were similarly tested as controls.

RESULTS

Table I presents the clinical study of eight patients. Seven of these seemed to have penicillin reactions, and the eighth (patient M. F.) probably represented a case of chronic dermatitis with penicillin considered by the family physician to be a possible factor. All the reactions occurred within eleven days following the last dose of penicillin. Penicillinase was administered within two days thereafter in four patients, within four days in two more, nine days later in one, and fifteen months later in patient M. F. Of the seven patients with definite penicillin reactions, two (D. L. and F. H.)

From the Department of Medicine, University of Wisconsin.
Received for publication Jan. 5, 1959.
*Torrance Medical Group, 1104 Sartori Ave., Torrance, California.

showed good to complete relief within twenty-four hours after penicillinase administration and required no further therapy, two more (R. C. and A. L.) had some improvement, and three (C. M., L. S., and S. H.) received no benefit. In none were second doses of penicillinase given. Two patients (A. L. and L. S.) had reactions consisting of chills and fever which appeared to be definitely related to the administration of penicillinase, and one (F. H.) had fever, the etiology of which was not clear, on the night of penicillinase administration.

TABLE I. CLINICAL DATA

PATIENT	PENICILLIN GIVEN	REACTION	ONSET OF REACTION P.C. LAST DOSE PENICILLIN	PENICILLINASE (800,000 U.) P.C. ONSET OF REACTION	RESULT	SIDE EFFECT
R. C.	Bicillin Feb. 15, 1958	Rash, arthralgia	11 days	1 day	Improved but still developed new erythematous areas	
C. M.	Duracillin Feb. 13, 15, 17, 18, 19, 1958	Urticaria, angioedema	10 days	2 days	Course unaltered; steroid needed	
D. L.	Duracillin 600,000 Feb. 26, 27, 28, 1958	Papular eruption, urticaria	5 days	2 days	Improved	
A. L.	Penicillin (Form K) 1,000,000 Feb. 2, 1958	Arthralgia, pruritus, urticaria	4 days	9 days	Better, but some new swelling next day	Chills and fever few hours later
F. H.	Procaine penicillin and streptomycin April 2, 1958	Urticaria	1 hour later	1½ hours	Cleared in 24 hours	Fever to 103° F. that night Etiology?
L. S.	Duracillin 600,000 Oct. 15, 17, 18, 1958	Urticaria, angioedema	7 days	3 days	No relief	Chills that night and following night
S. H.	Bicillin 1,200,000 July 31, 1958	Urticaria, angioedema	10 days	4 days	Increased angioedema; steroid given	
M. F.	Penicillin October, 1956	Dermatitis, nonallergic	11 days later	1½ years	No relief; Pase given diagnostically	

Table II presents data on the skin-test studies. Intradermal immediate wheal reactions were read in ten minutes and delayed reactions in twenty-four to forty-eight hours. It is noted that four patients (C. M., F. H., S. H., and M. F.) developed a positive immediate wheal reaction to penicillinase two weeks or more after therapy. In view of totally negative results in the control study, it seems reasonable to assume that in those patients (C. M.,

F. H., and M. F.) who had no tests prior to penicillinase therapy the positive reactions are valid evidence of injection-induced sensitivity. It is interesting to note that patients A. L. and S. H. showed positive reactions to penicillin, negative reactions to penicillinase, but still positive reactions to the mixture.

TABLE II. SKIN TEST RESULTS

TESTS A.C. AND P.C. PASE INJECTION	PATIENT							
	R. C.	C. M.	D. L.	A. L.	F. H.	L. S.	S. H.	M. F.
Penicillin 500 units per cubic centimeter								
A.C.								
Immediate*	Neg.					±	2+	
Delayed†	Neg.					Neg.	Neg.	
P.C.								
Immediate	Neg.	Neg.	Neg.	3+	1+	Neg.	2+	1+
Delayed	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	2 cm.	Neg.
Penicillinase 5,000 units per cubic centimeter								
A.C.								
Immediate	Neg.					Neg.	Neg.	
Delayed	Neg.					Neg.	1.5 cm.	
P.C.								
Immediate	Neg.	2+	±		2+	±	2+	3+
Delayed	Neg.	Neg.	Neg.		1 cm.	0.5 cm.	1.5 cm.	0
Penicillinase 10,000 units per cubic centimeter								
A.C.								
Immediate	Neg.					±	Neg.	
Delayed	Neg.					Neg.	2.5 cm.	
P.C.								
Immediate	Neg.	3+	±	Neg.	2+	±	2+	3+
Delayed	Neg.	Neg.	Neg.	Neg.	2.5 cm.	1.0 cm.	2 cm.	Neg.
Penicillin 500, penicillinase 5,000 units per cubic centimeter‡								
A.C.								
Immediate	Neg.					2+	1+	
Delayed	Neg.					Neg.	2 cm.	
P.C.								
Immediate	Neg.	2+	±	1+	1+	±	2+	Neg.
Delayed	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	2 cm.	Neg.
Penicillin 1,000, penicillinase 10,000 units per cubic centimeter‡								
A.C.								
Immediate	Neg.					2+	2+	
Delayed	Neg.					Neg.	0.5 cm.	
P.C.								
Immediate	Neg.	3+	±	3+	1+	2+	2+	2+
Delayed	Neg.	Neg.	Neg.	Neg.	1 cm.	Neg.	±	Neg.

*Immediate wheal tests were measured on size and shape of wheal.
†Delayed tests were measured in centimeters of induration.
‡Final concentration per cubic centimeter.

Of three patients who had pretreatment skin testing, one (S. H.) showed an initial positive delayed reaction to penicillinase while having a negative immediate wheal response, the significance of which cannot be definitely stated.

Ten normal patients, who were similarly tested, were all negative.

DISCUSSION

It has been proposed by various writers[1-3, 7, 12] that penicillinase has a definite place in the therapy of penicillin reactions. Becker[1] demonstrated that penicillinase will lower circulating penicillin to nondetectable levels and will continue to do so for four to seven days after administration. The same results have been obtained in experimental animals by Sureau[10] and Chen[5] and their colleagues.

Penicillinase, being a protein, is antigenic in its own right. As such, it should have the ability to produce antibodies having properties of both the neutralizing and the anaphylactic types.

Boyer and associates[4] demonstrated that pretreatment of guinea pigs with penicillinase appeared to stimulate "antipenicillinase" activity in the blood. In this study the animals were infected with a penicillin-sensitive organism and treated with penicillin, penicillin plus penicillinase premixed, penicillin and penicillinase given individually at separate sites, and penicillinase several days prior to the inoculation of bacteria. Inhibition of penicillin-protective effect on infection was demonstrable only where the penicillin and penicillinase were premixed. Survival of animals pretreated with penicillinase was greater than controls and this suggested the stimulation of antipenicillinase factors in the blood. Perlstein and Liebman[8-9] produced antipenicillinase precipitins in rabbits. This serum inhibited the destructive effect of penicillin in vitro. Tacking[11] confirmed the above and, in addition, demonstrated that rabbits immunized with penicillinase, subsequently infected with a penicillin-sensitive organism and a penicillinase-producing *Staphylococcus aureus,* and then treated with penicillin showed a 35 per cent mortality, as compared to 100 per cent for nonpenicillinase-immunized animals. Likewise, nonpenicillin-treated but immunized animals all died. Tacking's results demonstrate the sparing effect of penicillin by the neutralization of the bacteria-produced penicillinase by the antipenicillinase antibodies resulting from the prior immunization with penicillinase.

Fisher and co-workers[6] showed that all of thirty-six guinea pigs and nine rabbits developed sensitivity to penicillinase as demonstrated by circulating precipitins, agglutinins, passive cutaneous anaphylaxis, and positive skin tests.

In our limited human studies it appears that four out of eight patients developed positive skin sensitivity from a single injection of 800,000 units of penicillinase.

In theory, circulating penicillin in a sensitive host is the cause of the systemic allergic manifestation. By destroying the penicillin molecule, penicillinase theoretically inhibits further reaction. However, the demonstrated antigenicity of penicillinase in both human and animal experiments suggests caution in its use. The repeated injection of the drug under certain clinical conditions could result in serious anaphylactic reactions. Similarly, in addition to this potential hazard, the experimental demonstration of the development of antipenicillinase antibodies could well result in decreased effectiveness of

penicillinase activity. In some instances penicillinase fails to entirely destroy the antigenicity of penicillin in vitro (patients A. L. and S. H.) and this may occasionally explain therapeutic failure.

CONCLUSIONS

1. Penicillinase is an enzyme which causes the hydrolysis of penicillin to penicilloic acid. This material is devoid of antibacterial activity. The immunologic relationship of penicilloic acid to penicillin is not definitely known at this time.

2. Antibodies which inhibit penicillinase action have been reported experimentally.

3. Sensitizing antibodies are produced readily in animals and human beings. Repeated injections of penicillinase must be given with caution.

REFERENCES

1. Becker, R. M.: Effect of Penicillinase on Circulating Penicillin, New England J. Med. **254**: 952, 1956.
2. Becker, R. M.: Penicillinase Treatment of Penicillin Reactions. In Welch, H., and Martí-Ibañez, F. (editor): Antibiotics Annual 1957-1958, New York, 1958, Medical Encyclopedia, Inc., p. 310.
3. Becker, R. M.: A New Concept in the Treatment of Penicillin Reactions: Use of Penicillinase, Ann. Int. Med. **48**: 1228, 1958.
4. Boyer, F., Sureau, B., and Saviard, M.: Action of Penicillinase In Vivo. I. Treatment With Penicillin in Mice Who Were Inoculated With Streptococci and Who Were Previously Treated With Injections of Penicillinase, Ann. Inst. Pasteur **75**: 173, 1948.
5. Chen, J. Y., Bard, J. W., and Belsito, A. A.: In Vivo Penicillin Inactivating Effect and Toxicity of Penicillinase. In Welch, H., and Martí-Ibañez, F. (editors): Antibiotics Annual 1957-1958, New York, 1958, Medical Encyclopedia, Inc., p. 321.
6. Fisher, J. P., Cooke, R. A., Freedman, S. O., and Myers, P. A.: Experimental Sensitization With Penicillinase, J. ALLERGY **28**: 423, 1957.
7. Minno, A. M., and Davis, G. M.: Penicillinase in the Treatment of Penicillin Reactions, J. A. M. A. **165**: 222, 1957.
8. Perlstein, D., and Liebman, A. J.: The Production of Anti-Penicillinase Immune Sera, Science **102**: 197, 1945.
9. Perlstein, D., and Liebman, A. J.: The In Vitro Protection of Penicillin From Inactivation by Penicillinase, Science **102**: 174, 1945.
10. Sureau, B., Boyer, F., and Saviard, M.: Action of Penicillinase In Vivo. II. Study of the Concentration of Penicillin in the Blood of Rabbits Having Received Penicillinase, Ann. Inst. Pasteur **75**: 177, 1948.
11. Tacking, R.: Immunization Experiments With Penicillinase, Acta path. et microbiol. **36**: 61, 1955.
12. Zimmerman, M. C.: Penicillinase Treatment of Fifty-two Patients With Allergic Reactions to Penicillin. In Welch, H., and Martí-Ibañez, F.: Antibiotics Annual 1957-1958, New York, 1958, Medical Encyclopedia, Inc., p. 312.

The Effect of Heat Processing, Gamma Radiation, and Ultraviolet Radiation on the Anaphylactogenic Properties of Milk

H. F. Kraybill, Ph.D., M. S. Read, Ph.D., R. O. Linder, D.V.M., R. S. Harding, B.S., and G. J. Isaac, B.A., Denver, Colo.*

INTRODUCTION

DURING the past decade, much attention has been directed toward the use of ionizing radiation for the preservation of foods.[1] The effect of gamma irradiation or high-speed electrons on food proteins has been extensively explored by the use of both physical and chemical measurements.[2-6] However, little attention has been directed toward evaluating changes in biologic response or the anaphylactogenic properties of proteins.

As with heat treatment, irradiation of proteins produces denaturation, degradation, polymerization, and molecular rearrangement. These molecular alterations can be followed by changes in solubility, viscosity, absorption spectra, electrophoretic mobilities, and heat stability. Bellamy and colleagues[7, 8] noted viscosity changes in egg protein and casein, and Barron and Finkelstein[6] observed changes in sedimentation of bovine serum.

Lehman and Laug[9] proposed that the radiation-induced biochemical alteration of proteins could be traced biologically by a comparative evaluation of the antigenic properties of native and irradiated protein. Leone[10] measured the antigenic response of 1 per cent solutions of native and gamma-irradiated ovalbumin, the latter treated with an absorbed energy range of 268 to 344 electron volts per molecule (100 electron volts per molecule = 2.1×10^5 rads). Only 0.1 to 0.3 mcg. of irradiated protein nitrogen per animal was required for a mean sensitizing dose, whereas 2.5 mcg. of protein nitrogen per animal was required for the native protein.

As early as 1911, Doerr and Moldovan[11] found that ultraviolet radiation could reduce the antigenic activity of proteins. Using the light from a quartz mercury vapor lamp (½ kw.), Harris[12, 13] photo-oxidized plasma using

From the U. S. Army Medical Research and Nutrition Laboratory, Denver, Colorado.

Presented before the annual meeting of the American Academy of Allergy, Chicago, Illinois, Feb. 9-11, 1959.

These studies were supported under Contract No. DA-49-007-MD-549 with the office of the Surgeon General, Department of the Army, as a collaborative project between this laboratory and the University of Colorado. The opinions expressed in this publication are those of the authors and not necessarily those of the Department of the Army.

Received for publication Jan. 12, 1959.

*Present address: University of Colorado School of Medicine, Denver 20, Colorado.

hematoporphyrin as a photosensitizer. Within the range of 200 to 436 mμ, the absorption of ultraviolet energy by hematoporphyrin produced a forty fold increase in the oxygen uptake of plasma.

Continuous boiling of milk protein produces slight denaturation, reduces curd tension, and may alter the physical properties of the casein and whey proteins.[14, 15] Therefore, heat treatment has become a common practice in improving the digestibility of milk for infant feeding.

Wells and Osborne,[16] in their classical studies on the immunologic properties of proteins from milk, demonstrated that chemical constitution is related to the serologic properties of different proteins. Therefore, since hydrolytic and proteolytic processes degrade proteins into smaller molecular fragments, it is not surprising that these lack the antigenic properties characteristic of the native protein. It is the purpose of this study to investigate the effect of thermal processing, ultraviolet irradiation, and ionizing radiations on the anaphylactogenic properties of milk proteins.

MATERIALS AND METHODS

Preparation of Antimilk Serum.—Young rabbits weighing approximately 2.5 Kg. were injected with alum-precipitated milk proteins (0.5 ml. of raw skimmed milk, 0.3 ml. of physiologic saline, 0.1 ml. of 10 per cent potassium aluminum sulfate and 0.1 ml. of 0.5 N sodium hydroxide per dose). Daily subcutaneous injections were given on the first four days of the week for six consecutive weeks. Within one week following the last injection, the bleeding schedule was started; 35 ml. of blood was drawn daily for five days by cardiac puncture. On the sixth day, the animal was sacrificed by exsanguination. The sera were pooled and stored in serum bottles at 40° F. with 0.5 per cent phenol and 0.01 per cent Merthiolate as preservatives.

Guinea Pigs.—Male and female guinea pigs (Ziemet Bio-Farms, Madison, Wisconsin), each weighing approximately 280 to 320 grams, were used for the gross anaphylaxis measurements. For the Schultz-Dale uterine strip contraction measurements, similar young virgin females were employed.

Gamma Radiation of Raw Skimmed Milk.—Raw skimmed milk in screw-cap glass vials was packed and sealed in No. 2 metal cans, frozen, and then shipped with dry ice to the Gamma Irradiation Facility at the Materials Testing Reactor in Idaho Falls, Idaho. The gamma irradiation was provided by spent fuel rods (mixed fission products) in a water canal at ambient temperature. The dose rate was 2.88 to 3.38 $\times$ 10^6 rads per hour with an average energy of 0.75 Mev. The four groups of vials of milk were irradiated to total doses of 0.465, 2.79, 5.58, and 9.30 megarads, respectively. The cans were then repacked in dry ice and returned to our laboratory, where they were stored along with the control (nonirradiated) milk samples at –20° C.

Concurrent Radiation Distillation of Milk.—Samples of whole milk that had been concurrently irradiated and distilled were furnished for testing by the Department of Food Technology, Massachusetts Institute of Technology,

Cambridge, Massachusetts.[17] In this procedure, a vacuum evaporator is fitted into a cylindrical cobalt-60 source. Nitrogen is swept through the system, and sodium ascorbate is added to the milk to reduce off-flavor development. Volatile off-odor and off-flavor compounds are removed during irradiation and are collected in cold traps.

Ultraviolet Radiation of Milk.—Raw skimmed milk, placed in large Petri dishes and agitated with a magnetic stirrer, was irradiated with ultraviolet light from a Mineralite S-61 lamp with filter removed (wavelength 2,400 to 4,000 Å.). Samples were irradiated 13 cm. from the coils of the lamp for three hours, both with and without a photosensitizer (hematoporphyrin 100 mg. per 50 Gm. milk). After irradiation, the samples were stored at –20° C.

Heat-Treated Milk.—Screw-cap glass vials containing raw skimmed milk were inserted into a short length of close-fitting steel pipe which was sealed at both ends with threaded plugs. This unit was immersed in a hot oil bath which was thermoregulated to the desired temperature for thirty-five minutes. Eight heat-treated samples were prepared, having 10 degree temperature increments from 60° C. to 130° C. Samples were stored at –20° C.

Schultz-Dale Uterine Strip Measurements.—The Schultz-Dale apparatus and the technique for measurement of uterine strip contractions obtained by challenging sensitized uterine strips with histamine and milk proteins is essentially the method of Coulson.[18] The guinea pigs used in this work were passively sensitized by injecting 1.0 ml. of antimilk rabbit serum into the accessory cephalic vein approximately sixteen hours prior to challenging.

Gross Anaphylaxis Measurements.—Male and female guinea pigs were passively sensitized intraperitoneally with 0.5 ml. of antimilk rabbit serum. Forty-eight hours after sensitization, the animals were challenged with the processed milk or with the control untreated milk. The concentrations of the milk solutions ranged from 10 to 3,200 mcg. of protein N per 0.5 ml. injected into the accessory cephalic vein of the guinea pig. Symptoms and time of onset of anaphylactic response, as well as time of death, were noted in each case. The mean lethal shocking dose (LD_{50}) in terms of mcg. of protein N was calculated from the number of anaphylactic mortalities at each dose level. The method of probit analysis described by Finney[19] was used for this calculation.

Quantitative Electrophoretic Measurements.—The electrophoretic mobilities of the milk proteins from either control or gamma-irradiated samples were measured by the method of Durrum[20] and Block.[21] The casein and whey proteins were separated according to the method of Hipp and associates.[22]

Viscosity Measurements.—Viscosity measurements on the control and gamma-irradiated milk samples were made according to the method of McGoury and Mark.[23]

RESULTS

Comparative measurements of the contraction of sensitized uterine strip challenged with histamine or gamma-irradiated milk samples (Schultz-Dale technique) with the contractions for control or nonirradiated milk samples are

shown in Fig. 1. With the contraction for histamine or control raw milk used as the 100 per cent response, it is to be noted that 0.465 and 2.79 megarad radiation had little effect on molecular alteration of the milk protein. Higher radiation doses of 5.58 and 9.30 megarads produced contractions equalling 93.1 and 85.9 per cent of the contraction recorded for raw skimmed milk. After desensitizing the uterine strip with gamma-irradiated milk protein and making

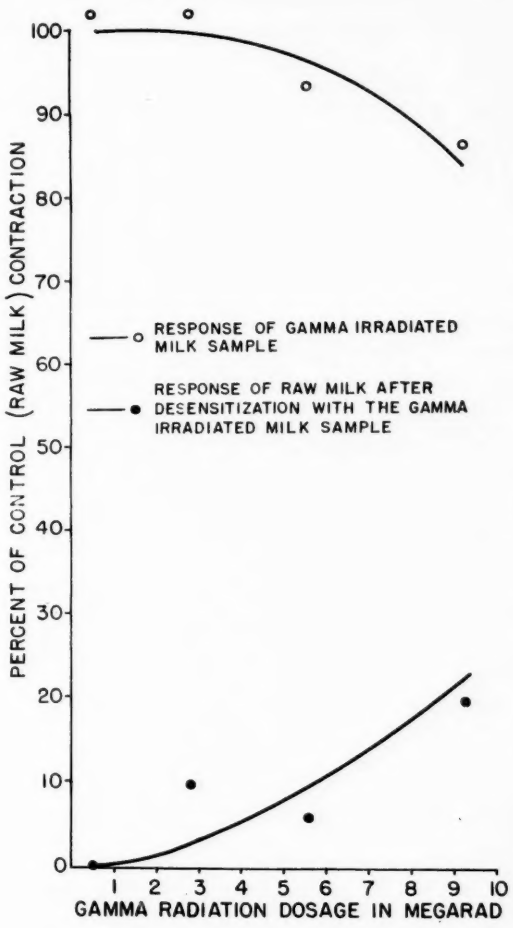


Fig. 1.—Antigenic response of guinea pig uterine tissue when challenged with milk proteins gamma irradiated at various dosage levels.

measurements on each sample at specific radiation dose, the cross-challenge response with raw milk protein produced contractions ranging only from 0 to 19.4 per cent, as shown in the lower curve in Fig. 1. Again, the observations reveal that significant changes in the milk protein molecule require a radiation dose of 5.58 megarads or greater.

Values obtained for the mean lethal shocking doses (LD_{50}) in the gross anaphylaxis experiments show similar trends. For example, the LD_{50} value in Table I for control raw milk was 36 ± 7 meg. of protein nitrogen, whereas the

9.30 megarad sample produced an LD_{50} value of 1,157 ± 422 mcg. of protein nitrogen. Intermediate values of 64 ± 11 mcg. of protein nitrogen for the 2.79 megarad treatment and 602 ± 270 mcg. of protein nitrogen for the 5.58 megarad dose indicate quite clearly that increasing the level of radiation altered the biologic response to these radiation-modified milk protein solutions. The concurrent radiation-distilled milk, which received a 9.30 megarad dose, did not yield an LD_{50} value comparable to that obtained with the gamma-irradiated sample (Table I). It is possible that removal of the smaller molecular

TABLE I. MEAN LETHAL SHOCKING DOSE (LD_{50}) FOR MILK-SENSITIZED GUINEA PIGS CHALLENGED WITH GAMMA-IRRADIATED, CONCURRENT RADIATION-DISTILLED,* AND RAW SKIMMED MILK

SERIES	RADIATION DOSE 10^6 RADS	NUMBER OF GUINEA PIGS	LD_{50} MCG. PROTEIN NITROGEN	APPROXIMATE RATIO CONTROL VS. IRRADIATED
1	0	66	36 ± 7	1:0.8
	0.475	66	29 ± 4	
2	0	77	32 ± 4	1:2
	2.79	87	64 ± 11	
3	0	64	69 ± 16†	1:9
	5.58	50	602 ± 270	
4	0	60	37 ± 4	1:31
	9.30	55	1,157 ± 422	
		Values for Concurrent Radiation-Distilled Milk		
5	0	68	25 ± 4	1:5
	9.30	71	131 ± 23	

*Whole milk irradiated with cobalt-60 source and concurrently distilled for removal of volatile off-flavor, off-odor compounds.

†Stock of antisera had lower antibody titer.

fragments by distillation prevented further interaction with the milk protein. The net effect would be decreased protein change and, consequently, an increased anaphylactic response. Such an increase was obtained in our work.

TABLE II. MEAN LETHAL SHOCKING DOSE (LD_{50}) FOR MILK-SENSITIZED GUINEA PIGS CHALLENGED WITH HEAT-TREATED, ULTRAVIOLET-IRRADIATED, AND RAW SKIMMED MILK

SERIES	TREATMENT	NUMBER OF GUINEA PIGS	LD_{50} MCG. PROTEIN NITROGEN	APPROXIMATE RATIO CONTROL VS. TREATED SAMPLE
1	Control	55	24 ± 3	1:14
	90° C.	55	347 ± 99	
2	Control	55	27 ± 3	1:8
	UVN	53	228 ± 52	
3	Control	55	26 ± 4	1:49
	UVS	81	1,272 ± 299	

UVN = Raw skimmed milk irradiated three hours with ultraviolet light without filter and with no photosensitizer.

UVS = Raw skimmed milk irradiated three hours with ultraviolet light without filter and with hematoporphyrin as photosensitizer.

Heat processing of milk also modified the biologic response observed in the contractions of milk-sensitized uterine strips. Milk processed at 60° C. produced, on initial challenge, a response similar to that for raw milk (Fig. 2). The 130° C. sample, however, exhibited a drastic alteration in that the initial mean response was only 19.8 per cent of that for the control milk and the mean cross-challenge response was 99.9 per cent. Other heat-treated samples (70° C.

to 120° C.) showed intermediate values between these two extremes. Similarly, the LD_{50} values (Table II) indicate that an elevation of processing temperature to 90° C. had a pronounced effect on biologic response. The increase from 24 ± 3 mcg. of protein nitrogen to 347 ± 99 mcg. of protein nitrogen is highly significant, the ratio between the control and 90° C. values being 1 to 14.

The addition of a photosensitizer, such as ferrous sulfate or hematoporphyrin, to the milk accelerated the photochemical effect of ultraviolet light on the proteins. This effect is clearly demonstrated by the LD_{50} data in Table II.

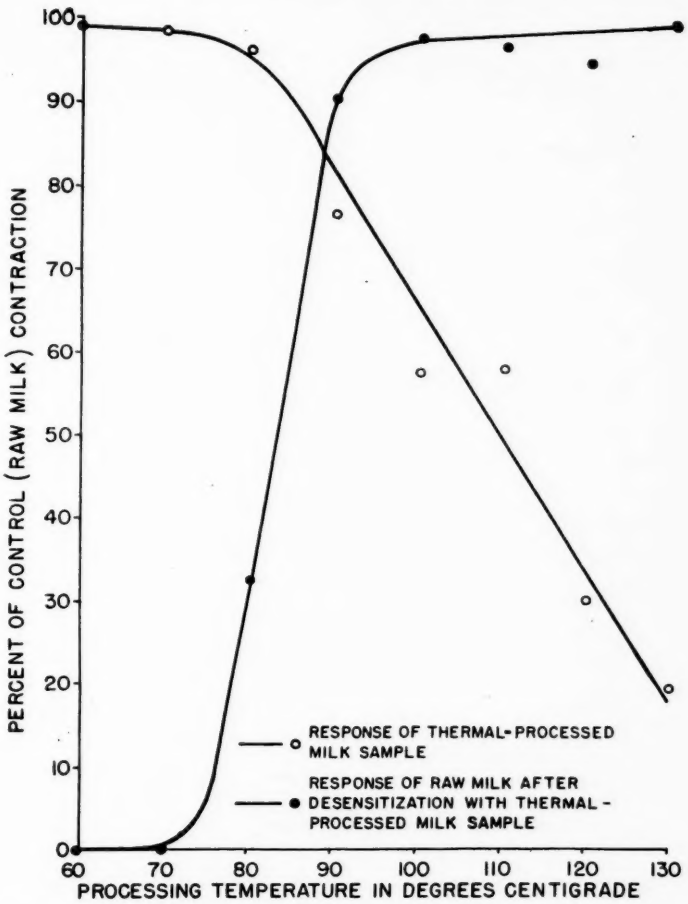


Fig. 2.—Antigenic response of guinea pig uterine tissue when challenged with milk proteins heat treated thirty-five minutes at various temperatures.

The LD_{50} value of the ultraviolet-treated milk with photosensitizer was 1,272 ± 299 mcg. of protein nitrogen, which is almost six times that of 228 ± 52 mcg. of protein nitrogen for the milk protein without photosensitizer added. It is of interest that ultraviolet light, acting on milk protein without a photochemical activator, will produce significant protein change. For instance, the LD_{50} value for sample UVN (Table II) was eight times that of its control.

The extent of the modification of milk protein by various processing methods, as shown by alteration in biologic response, has been confirmed by physical and chemical measurements. For example, the alteration in the electrophoretic patterns of the gamma-irradiated whey proteins (Fig. 3) demonstrates the effect of increasing the radiation dose. Further evidence of these molecular alterations was obtained from viscosity measurements. The increase in viscosity with radiation dose (Fig. 4) reflects the denaturation of protein by radiation into smaller fragments which, in turn, aggregate to form heavier, more viscous polymers.

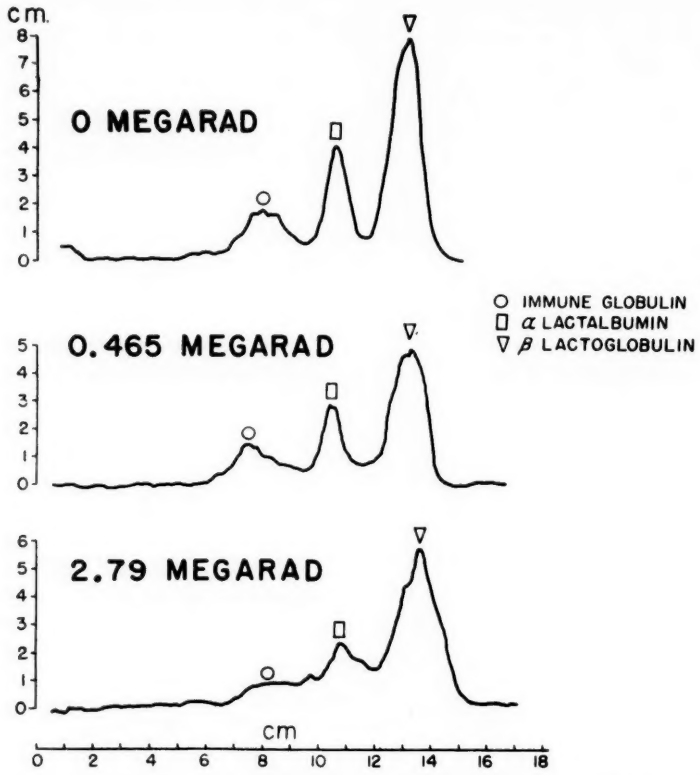


Fig. 3.—Effect of gamma radiation on the electrophoretic pattern of whey proteins.

DISCUSSION

General comparisons of the altered antigenic response caused by gamma and ultraviolet irradiation and thermal processing can be made by either the Schultz-Dale uterine strip technique or gross anaphylaxis experiments. Although the curves in Figs. 1 and 2 have but a few points with high standard deviations, comparisons between the thermal and the gamma-radiation treatments may be extracted. For example, the 9.30 megarad sample had an antigenic response approximating that for an 89° C. heat-treated milk. Similarly, a 5.58 megarad sample was approximately equal in antigenic response to a milk solution processed at 77° C.

The data obtained in the gross anaphylaxis experiments do not quite parallel the calculated values obtained by the Schultz-Dale method. By gross anaphylaxis measurements (Table II), the 90° C. sample has a value which is roughly equivalent to the 5.58 megarad sample (Table I). It would thus appear that the methods are not exactly comparable in estimating biologically the extent of molecular alteration of protein.

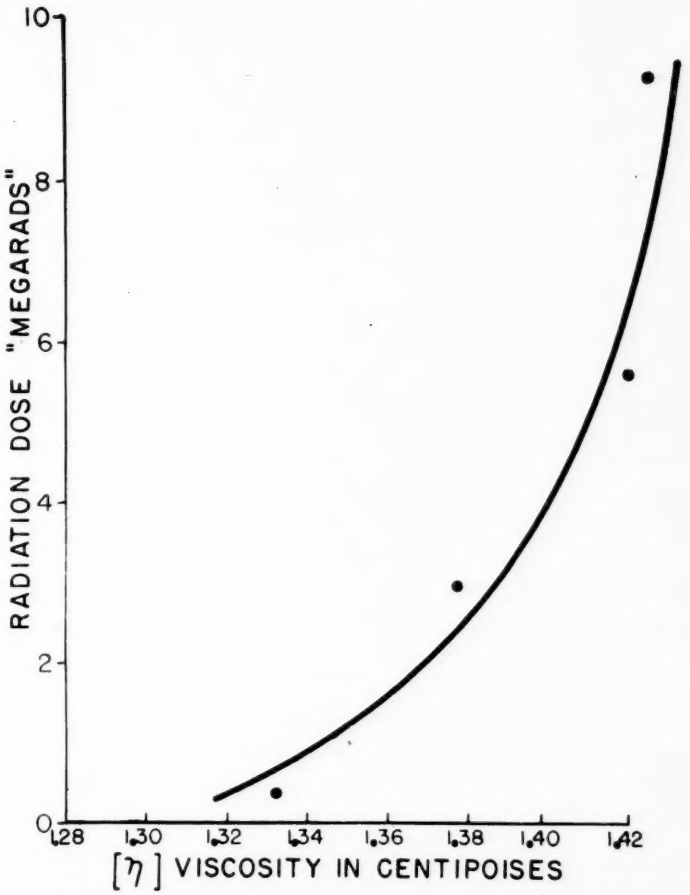


Fig. 4.—Effect of gamma radiation on viscosity of milk proteins.

The photo-oxidation of milk protein by ultraviolet irradiation in the presence of a photosensitizer effectively alters the biologic response to milk (Table II). It would appear from a comparison of the LD_{50} values for the 9.30 megarad sample (Table I) and the UVS sample (Table II) that, under the conditions by which we operated, the ultraviolet treatment altered the allergenicity more than the 9.30 megarad radiation dose. Our data clearly indicate, however, that a photosensitizer is required for effective alteration of milk protein by ultraviolet light (see samples UVN and UVS, Table II). It is

believed that hematoporphyrin activates the aromatic structure in phenylalanine, tyrosine, and tryptophane under the influence of ultraviolet light.[13] This reaction proceeds rapidly, resulting in molecular rearrangement.

The changes which we have obtained in the electrophoretic patterns and viscosity of irradiated milk provide, in part, an explanation for the altered biologic responses which have been observed. Further work is needed, however, in evaluating the chemical changes induced in milk protein by gamma and ultraviolet irradiation as well as thermal treatment. Profitable studies employing the different fractions of whey and casein could be undertaken. The anaphylactogenic properties of these fractions also could be investigated.

Heat processing seems to be equally as efficient as radiation processing for modifying the allergenicity of milk protein. Gamma irradiation of milk produces some browning of the product, with the development of certain off-odor and off-flavor components. However, improvements in technology, such as concurrent radiation distillation, can provide a satisfactory product.

Perhaps a combination of ionizing radiation, heat, and ultraviolet irradiation could be employed in altering the anaphylactogenic properties of food products such as milk. In any case, clinical studies with human subjects who are allergic to milk protein would seem to be warranted on the basis of these investigations.

SUMMARY

Molecular alteration in proteins has been accomplished with extensive heat treatment, ultraviolet irradiation in the absence or presence of photosensitizer, and gamma radiation. Alteration in the anaphylactogenic properties of raw skimmed milk protein, when tested on milk-sensitized guinea pigs, was observed by all three methods. Measurements made of the contractions on excised uterine strip from milk-sensitized guinea pigs produced by a challenge with gamma-irradiated milk revealed that a 9.30 megarad sample produced a response which was 85 per cent of that produced by raw skimmed milk. A lower radiation dose of 5.58 megarads produced a contraction which was 93 per cent of that produced by raw skimmed milk.

The mean lethal shocking doses (LD_{50} values) for gamma-irradiated milk samples at radiation doses of 0.456, 2.79, 5.58, and 9.30 megarads were, respectively, 29 ± 4, 64 ± 11, 602 ± 270, and 1,157 ± 422 mcg. of protein nitrogen when injected into milk-sensitized guinea pigs. The LD_{50} value for 90° C. milk heated for thirty-five minutes was 347 ± 99 mcg. protein nitrogen. The LD_{50} value for ultraviolet-irradiated milk without photosensitizer (hematoporphyrin) was 228 ± 52, and with hematoporphyrin added it was 1,272 ± 299 mcg. of protein nitrogen. The LD_{50} value for control or raw skimmed milk in all experiments ranged from 24 to 37 mcg. of protein nitrogen.

Using treatment-response curves, 9.30 megarad gamma-irradiated milk was found to be equivalent to an 89° C. heat-treated milk.

Electrophoretic patterns and viscosity measurements have been made on gamma-irradiated milk protein.

The significance of the three methods of processing on the alteration of the allergenicity of milk protein has been discussed.

The authors wish to acknowledge the suggestions and assistance of the following: Dr. E. J. Coulson, Allergens Section, U. S. Department of Agriculture, Agricultural Research Service, Washington, D. C.; Dr. Arnold H. Johnson, National Dairy Research Laboratories, Inc., Oakdale, New York; and Dr. S. A. Goldblith, Department of Food Technology, Massachusetts Institute of Technology, for supplying concurrent radiation-distilled whole milk samples. The technical assistance of Mr. T. Michael Shaw and M/Sgt. R. P. vanAlmen is gratefully acknowledged.

REFERENCES

1. Bailey, S. D., Davies, J. M., Morgan, B. H., Pomerantz, R., Siu, R. G. H., and Tischer, R. G.: Radiation Preservation of Food, Washington, 1957, U. S. Army Quartermaster Corps and U. S. Government Printing Office.
2. Bailey, S. D., Davies, J. M., Morgan, B. H., Pomerantz, R., Siu, R. G. H., and Tischer, R. G.: Radiation Preservation of Food, Washington, 1957, U. S. Army Quartermaster Corps and U. S. Government Printing Office, pp. 133-158.
3. Ginger, I. D., Lewis, U. J., and Schweigert, B. S.: Changes Associated With Irradiating Meat and Meat Extracts With Gamma Rays, J. Agr. Food Chem. **3**: 156-159, 1955.
4. Kirn, J. F., Urbain, W. M., and Czarnecki, H. J.: Characteristics of Electron Irradiated Meat Stored at Refrigerator Temperatures, Food Tech. **10**: 601-603, 1956.
5. Marbach, E. P., and Doty, D. M.: Sulfides Released From Gamma Irradiated Meat as Estimated by Condensation With *N, N*-Dimethyl-p-phenylenediamine, J. Agr. Food Chem. **4**: 881-884, 1956.
6. Barron, E. S. G., and Finkelstein, P.: Studies on the Mechanism of Action of Ionizing Radiations. X. Effect of X-rays on Some Physicochemical Properties of Proteins, Arch. Biochem. **41**: 212-232, 1952.
7. Bellamy, W. D., Pultz, W., Mendenhall, R. M., and Clark, L. B.: Viscosity Changes in Electron Irradiated Proteins, Radiation Research **5**: 469-470, 1956.
8. Bellamy, W. D., Pultz, W., Mendenhall, R. M., and Clark, L. B.: Solubility Studies on Electron Irradiated Proteins, Radiation Research **5**: 490, 1956.
9. Lehman, A. J., and Laug, E. P.: Evaluating the Safety of Radiation Sterilized Foods, Nucleonics **12**: 52-54, 1954.
10. Leone, C. A.: Alteration of Antigenicity of Ovalbumin by Gamma Rays, Federation Proc. **16**: 422, Abstract 1809, 1957.
11. Doerr, R., and Moldovan, J.: Die Wirkung des Ultravioletten Lichtes auf das Eiweissantigen und zeinen antikörper, Wien. klin. Wchnschr. **24**: 555-559, 1911.
12. Harris, D. T.: Photo-oxidation of Plasma; a Note on Its Sensitization, Biochem. J. **20**: 280-287, 1926.
13. Harris, D. T.: Observations on the Velocity of the Photosensitization of Proteins and Amino Acids, Biochem. J. **20**: 288-292, 1926.
14. Palmer, L. F.: Effect of Heat on Calcium Salts and Rennet Coagulability of Cow's Milk, Proc. Soc. Exper. Biol. & Med. **19**: 137-142, 1931.
15. Sullivan, R. A., Hollis, R. A., and Stanton, E. K.: Sedimentation of Milk Proteins From Heated Milk, J. Dairy Sc. **40**: 830-833, 1957.
16. Wells, H. G., and Osborne, T. B.: Anaphylaxis Reactions With Proteins From Milk, J. Infect. Dis. **29**: 200-216, 1921.
17. Wertheim, J. H., Roychoudhury, R. N., Hoff, J., Goldblith, S. A., and Proctor, B. E.: Irradiation Preservation of Milk and Milk Products, J. Agr. Food Chem. **5**: 944-950, 1957.
18. Coulson, E. J.: The Schultz-Dale Technique, J. ALLERGY **24**: 458-473, 1953.
19. Finney, D. J.: Probit Analysis, Cambridge, England, 1947, The University Press.
20. Durrum, E. L.: Microelectrophoretic and Microionophoretic Technique, J. Am. Chem. Soc. **72**: 2943-2948, 1950.
21. Block, R. J., Durrum, E. L., and Zweig, G. A.: A Manual of Paper Chromatography and Paper Electrophoresis, New York, 1958, Academic Press, Inc., pp. 523-532.
22. Hipp, N. L., Groves, M. L., Custer, J. H., and McMeekin, T. L.: Separation of Alpha Casein, J. Am. Chem. Soc. **72**: 4928-4931, 1950.
23. McGoury, T. E., and Mark, H.: Physical Methods of Organic Chemistry; Determination of Viscosity, New York, 1949, Interscience Publishers, Inc., Chap. VIII.

Studies on the Antigenic Relationship of Insect Extracts by Reagin Neutralization

Evidence for Nonspecific Inactivation of Reagin

Jacob J. Pruzansky, Ph.D., Samuel M. Feinberg, M.D., Arthur Kaminker, M.D., and Leo N. Meleyco, M.D., Chicago, Ill.

CLINICAL observations have indicated that the dust of disintegrated insects might be an important cause of inhalant allergy.[1] A report has previously been made of the antigenic relationships of extracts of several insects; in that study we employed hemagglutination and gel diffusion.[2] In attempting to demonstrate allergenic relationships between insects by passive transfer, we made certain observations which led to a more critical study of the mechanism of desensitization of passively sensitized skin sites. As a result of these investigations, some of the limitations of this method, previously observed in this laboratory,[3] were confirmed and amplified, and evidence was obtained that a specific allergen-reagin reaction in the skin inactivated unrelated reagins that were present in the same site. Nonspecific wheals caused by histamine or compound 48/80 likewise resulted in apparent destruction of reagins at the site of reaction.

Bowman and Walzer[4] have also reported that wheals produced by immunologic or nonimmunologic means would result in hyporesponsiveness of previously sensitized skin sites. Since tissue unresponsiveness to histamine was only temporary, they concluded, apparently by the process of elimination, that the irreversible hyporesponsiveness to allergens was due to reagin destruction. In the experiments reported here, it has been established experimentally that unrelated reagins were inactivated by an allergen-reagin reaction in the skin.

METHODS

Serum containing reagins to several insects, molds, and ragweed was mixed with an equal volume of buffered diluent. Intradermal injections of 0.05 ml. of the diluted serum were made into multiple sites on the arms and backs of nonatopic persons whose skin did not react to 1:10,000 dilutions of the allergens to

From the Allergy Research Laboratory, Department of Medicine, Northwestern University Medical School.

This investigation was supported in part by Research Grant E-1226 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service, and Asthmatic Children's Aid, Chicago, Illinois.

Presented at the fifteenth annual meeting of the American Academy of Allergy, Chicago, Illinois, Feb. 9-11, 1959.

Received for publication Jan. 12, 1959.

be tested. Forty-eight hours later sensitized sites on the arms were titrated with 0.01 ml. of threefold dilutions of the test allergens, histamine or compound 48/80. The highest dilution producing a ++ to +++ reaction was selected for routine desensitization and subsequent testing, since this is the range of reactivity most sensitive to variations in experimental conditions. The reactions on testing were recorded as follows: +, erythema diameter less than 2 cm.; ++, a wheal of up to 3 mm. in diameter; +++, a 3 to 5 mm. wheal; ++++, a wheal of more than 5 mm.

Specific desensitization was accomplished by daily injections of 0.1 ml. of the selected dilution of allergen extract, the process being considered complete when a test dose of 0.01 ml. of the same dilution produced no reaction. This usually required two injections.* After desensitization was completed, a further 0.1 ml. of allergen was injected. Histamine and compound 48/80 were used to inactivate sites nonspecifically in the same way as described for allergen. At various times after the final inactivating injection, sites were challenged with 0.01 ml. doses of the selected dilutions of reagin or pharmacologic agent.

RESULTS

Previous work indicated that human skin might react normally again one week after depletion with compound 48/80. Several nonatopic persons were sensitized, depleted, and cross-tested eight days later. These preliminary experiments showed that all sites were hyporesponsive, regardless of whether they were tested with homologous or heterologous extracts or with histamine or compound 48/80. An extended time series was therefore planned to allow delineation of the specific and nonspecific phases of the reaction.

Three subjects were passively sensitized in eight vertical columns of eight sites each on the back and on several sites on the arms. Titration was carried out on the arm sites, and dilutions were selected as described earlier. Desensitization was then carried out as follows: rows 1 and 2, control sites, were not desensitized; rows 3, 4, and 5 were desensitized with alternaria extract; rows 6 and 7 with compound 48/80; and row 8 with histamine. Starting on the day after depletion and at various times thereafter, sites were tested as follows: row 3, cricket extract; row 4, compound 48/80; row 5, histamine; row 6, cricket extract; row 7, compound 48/80; and row 8, cricket extract. After two or three weeks some control reactions had decreased in intensity. The next stronger solution of extract was found to produce a ++ or +++ reaction and was then used for testing.

The data obtained in one individual case are presented in Table I. The other two subjects reacted in an almost identical fashion. A definite time pattern can be traced. At first all skin reactivity was virtually abolished. In two to three weeks, sites depleted by any means became responsive to either histamine or compound 48/80, but completely normal reactivity was not usually observed for about twenty-one days. It is to be noted, in contrast, that there was a

*The sites thus desensitized were still partially reactive to histamine.

persistent impairment of response (up to seven weeks) to allergen in sites previously made unresponsive by immunologic (allergen) or nonimmunologic (compound 48/80 and histamine) means. The sites were not tested later than fifty-one days after inactivation, since by that time control sensitized sites had become much less responsive.

TABLE I. TESTING OF PASSIVELY SENSITIZED SKIN SITES AT VARIOUS TIMES AFTER INACTIVATION

	SITES INACTIVATED WITH:					
	ALTERNARIA	ALTERNARIA	ALTERNARIA	COMPOUND 48/80	COMPOUND 48/80	HISTAMINE
	THEN TESTED WITH:					
TIME AFTER INACTIVATION	CRICKET	COMPOUND 48/80	HISTAMINE	CRICKET	COMPOUND 48/80	CRICKET
One day	0	0	1+	0	0	0
One week	0	0	1+	0	0	0
Two weeks	0	1+	2+	0	1+	1+
Three weeks	0	3+	3+	0	3+	0
Four weeks	0	3+	3+	0	3+	1+
Five weeks	0	3+	2+	0	3+	1+
Six weeks	0	2+	2+	0	2+	*
Seven weeks	0	3+	3+	0	3+	0

*Technical error.

In three weeks, when tissue responsiveness had become normal, and at weekly intervals, some of the inactive sites were resensitized with the original reaginic serum and tested forty-eight hours later with allergen. Reactions of ++ to +++ were observed in each case. Similarly inactive sites injected with normal human serum did not respond to allergen. In this way it was demonstrated that reagin had been the only limiting factor in the "permanently" unresponsive sites.

DISCUSSION

The immunologic whealing response can conveniently be considered to occur in several steps: (1) allergen-reagin combination; (2) activation of mediator release mechanism; (3) release of mediator, if available; and (4) tissue response to released mediator. No skin reaction will be observed if any of these processes is inhibited. Shortly after desensitization, and for ten to twenty-one days, there was a reduced response to either histamine or compound 48/80 on any pretreated site. It seems likely that the initial hyporesponsiveness was due to both the absence of available mediator and a refractoriness of tissue to any mediator that might have been released. After this time the sites were fully resupplied with mediator, and the responsiveness to mediator was again normal. In spite of this, all the variously depleted sites were still unresponsive to allergen. According to the above formulation, this persistent hyporesponsiveness could have been due only to either lack of allergen-reagin reaction or inhibition of the mediator release mechanism. Restoration of normal skin response to allergen after resensitizing the variously desensitized refractory sites demonstrated that the

original reagins had in some way been made unavailable for reaction with allergen. This, of course, does not point to the actual mechanism of inactivation of the reagin.

Several implications of these findings are of interest. The reliability of the passive transfer method using intradermal desensitizing injections for the study of allergenic relationship must be questioned. This criticism may not apply to the test tube "neutralization" technique. Antihistamines injected locally may help reduce wheal formation during desensitization of passively sensitized sites. The effectiveness of locally injected antihistamine on various whealing reactions has been discussed.[5] Siegel, Bowman, and Walzer[6] showed that antihistamines prevented the local reaction due to the sensitizing serum itself by addition to the serum of appropriate amounts of antihistamine. Our experience is that complete abolition of reactivity during desensitization would be very difficult to attain. At present there is being evolved a method for rendering sites temporarily unresponsive before sensitization and specific desensitization. Cross-testing is then delayed until normal tissue response is restored. These studies will be the subject of a future report.

The findings in the experiments reported here suggest other approaches to the study of the mechanism of the allergen-reagin reaction. The removal of reaginic activity by an extraneous allergen-reagin system or by a nonspecific agent, such as compound 48/80, must also suggest the possibility of a means of therapy. Exhausting a localized area of an undesirable symptom-producing reagin might possibly be achieved by such means, even if the allergen producing the symptoms were not known. The removal of reagins from the body as a whole, of course, is far less likely to be successful. Any of the suggested procedures would probably require continued treatment, since reagin is continuously being produced in actively sensitized persons.

SUMMARY

1. Skin sites sensitized with serum containing multiple unrelated reagins and desensitized with one allergen or treated with compound 48/80 fail to react to compound 48/80 or histamine for ten to twenty-one days.

2. Testing such sites with an unrelated allergen shows that all reaginic activity has been permanently lost at these sites.

3. That such loss is not due to tissue injury is demonstrated by the fact that the sites may be made reactive again after twenty-one days by resensitization with the original reaginic serum.

4. These findings emphasize the pitfalls inherent in some of the techniques of passive transfer in the study of allergen relationships. They also suggest certain other approaches to the study of antigen and antibody and a possible approach to therapy in some types of allergy.

REFERENCES

1. Feinberg, A. R., Feinberg, S. M., and Benaim-Pinto, C.: Asthma and Rhinitis From Insect Allergens. I. Clinical Importance, J. ALLERGY **27**: 437, 1956.
2. Pruzansky, J., Feinberg, A. R., Schick, G., and Feinberg, S. M.: Antigenic Relationships in Insect Extracts, Proc. Soc. Exper. Biol. & Med. **97**: 312, 1958.

3. Feinberg, S. M., Feinberg, A. R., Rebhun, J., and Malkiel, S.: Liberation and Depletion of Histamine From Human Skin, Quart. Bull. Northwestern Univ. M. School **28:** 246, 1954.
4. Bowman, K. L., and Walzer, M.: Studies in Reaginic and Histamine Wheals. I. The Effects of Reaginic and Histaminic Wheals Upon the Subsequent Responsiveness of Passively Sensitized Cutaneous Sites, J. ALLERGY **24:** 126, 1953.
5. Friedlaender, S., and Feinberg, S. M.: Histamine Antagonists. III. The Effect of Oral and Local Use of β-Dimethylaminoethyl Benzhydryl Ether Hydrochloride on the Whealing Due to Histamine, Antigen-Antibody Reactions and Other Whealing Mechanisms; Therapeutic Results in Allergic Manifestations, J. ALLERGY **17:** 129, 1946.
6. Siegel, B., Bowman, K. L., and Walzer, M.: Studies in Reaginic and Histaminic Wheals. II. The Effect of Locally Administered Antihistamine on the Passive Transfer Reaction, J. ALLERGY **24:** 212, 1953.

High-Efficiency Pollen Samplers for Use in Clinical Allergy

J. B. Harrington, M.A., G. C. Gill, M.A., and B. R. Warr, M.S., Ann Arbor, Mich.

INTRODUCTION

INTEREST in the particulate population of the atmosphere is common to personnel in many scientific fields, including the botanist, the plant pathologist, the cloud physicist, the medical allergist, the public health official, and the military and civilian air pollution expert. These scientists are faced with the very difficult problem of attempting to measure particulate concentrations in a turbulent atmosphere.

The same problem has been encountered by the staff of the Meteorological Laboratories of the University of Michigan in the course of interdisciplinary studies carried out by a research team of botanists, allergists, public health statisticians and meteorologists. A sampler which would collect ragweed pollen (20 microns in diameter) efficiently and which would be inexpensive enough to use in mass sampling programs was needed.

The meteorological group has at its disposal a closed-circuit wind tunnel, which was used during the spring of 1958 to measure the efficiencies of several of the common sampling techniques. The results were so definitive that the investigation was continued both in the tunnel and out of doors. New sampling techniques were developed and tested; two of these proved to be much more efficient than the standard techniques in use.

SAMPLING THEORY

The efficiency of a sampler will depend upon the following factors:

1. The ability to sample air with the same particulate concentrations that exist in the environmental air.

2. The success in retaining the particle, once it has touched the collecting surface.

The second factor has not been considered in calculations of concentration or efficiency throughout this article unless specifically mentioned.

It may be worth while to review briefly the various techniques used in air sampling and to indicate their limitations in a turbulent atmosphere. Most may be included in one or another of the following classifications:

From the Meteorological Laboratories of the Department of Civil Engineering, University of Michigan College of Engineering.

Publication No. 12 on *Atmospheric Pollution by Aeroallergens* under research grant No. E-1379(c) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

1. Drawing a known volume of air through a filter.

2. Impinging a known volume of air on a suitable collecting surface.

3. Precipitating particles thermally or electrostatically from a known volume.

4. Allowing particles of known fall rate to settle on a measured area.

VOLUMETRIC SAMPLERS

The filters, the precipitators, and several samplers of the impingement type draw air into the sampler through an aperture. Unless the air enters the aperture isokinetically, that is, without acceleration or deceleration, the collection will not represent the true concentration. For example, suppose a

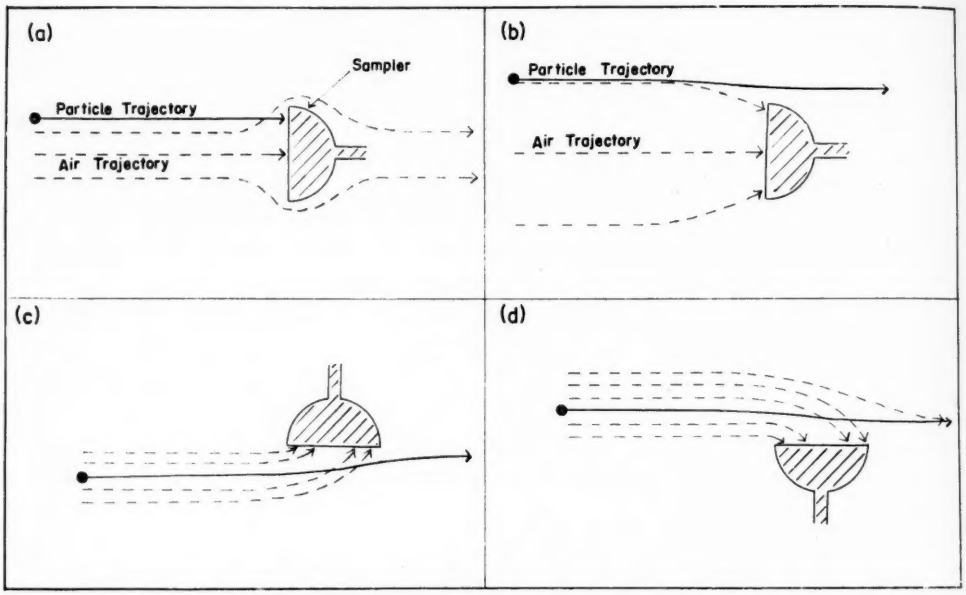


Fig. 1.—Schematic diagram showing the effect of particle inertia on the collection efficiency of aperture-type samplers in a moving airstream. *A*, Sampler facing into a wind of higher speed than the flow into the sampler. *B*, Sampler facing into a wind of lower speed than the flow into the sampler. *C* and *D*, Sampler facing normal to the wind.

filter sampler is oriented into the wind. When the wind is stronger than the rate of flow through the filter, particles will cross the streamlines due to their inertia and impinge on the filter, producing an unreasonably large catch (Fig. 1, *A*). Under the same conditions, if the wind is slower than the flow into the fllter, the catch will be too low (Fig. 1, *B*). When the sampler is mounted with the aperture downward or facing normal to the flow, the effect of particle inertia is even larger and the catch will be exceptionally low (Fig. 1, *C* and *D*). Experimental confirmation of these very important factors is given later in this article.

It cannot be assumed that the wind flow is as simple as described above. Turbulent eddies create rapid fluctuations in wind direction, making it impossible to keep a sampler oriented into the wind. The same eddies cause rapid fluctuations in wind speed, which make it impossible to sample isokinetically. Moreover, vertical gusts carry particulates into upward- or downward-facing apertures, increasing the collection. Unfortunately, there is no way to separate the collection due to the volumetric sample from the collection due directly to turbulent impingement.

In addition to the inertia, the drag coefficient of the particle being sampled has an important bearing on collection efficiency. Very small particles have relatively small inertias and large drag coefficients; hence, they tend to follow the streamlines better than large particles and are therefore sampled more efficiently. Unfortunately, most particles of importance as aeroallergens are 13 to 35 microns in diameter and have relatively large inertias.

At very low wind speeds and with small flow rates into the sampler, particle acceleration will be minimized, and reasonably high collection efficiency should result. For this reason, the type of sampler in which air is drawn through an aperture should be effective indoors, and also outdoors when the winds are very light.

GRAVITATIONAL SAMPLERS

The gravitational sampler can be used to measure particulate concentration only if it can be assumed that the particle falls at a constant rate V_f through the air. When this is the case, the concentration may be expressed as follows:

$$\chi = \frac{C}{V_f A t},$$

where C is the number of particles collected,
A is the area of a horizontal sampling surface,
χ is the volumetric concentration of the particulate, and
t is time.

Durham[4] and others have pointed out that, because of the large vertical motions associated with atmospheric turbulence, the fall rate cannot be assumed to be constant. Even a 1 m.p.h. wind will produce vertical velocities in the lowest 20 feet of the atmosphere of the order of 4.5 cm. per second under normal wind conditions.[9] These velocities are large compared to the average fall rate for most pollen or spores. For instance, low annual ragweed pollen falls at about 1.6 cm. per second according to Crawford[3]—just one-third of the vertical gust speeds that occur with a 1 m.p.h. wind. Wind speeds of 10 m.p.h. reduce this ratio to one-thirtieth. This means that under all but the calmest conditions more pollen will be brought into contact with the sampler through turbulent eddies than through gravitational settling.

Wind-tunnel tests showed that there is another even more important objection to the use of the "gravity slide" method of air sampling. When wind moves past a horizontal slide, the stream is split by the edge of the slide, forming

a thin laminar boundary layer between the slide and the free stream (Fig. 2). Most particulates will bypass the slide, although eddies in the free stream may carry some particles into the laminar boundary layer where they may settle onto the slide.

The orientation of the sampler has an important bearing on the catch. Slides whose long axes are parallel to the wind catch ten to 100 times more pollen than those whose short axes are in the direction of the wind. The reason for this effect is probably that when the long axis of the slide is parallel to the flow, pollen has three times as much time to fall through the laminar layer onto the slide. Both outdoor and wind-tunnel experiments confirm this result.

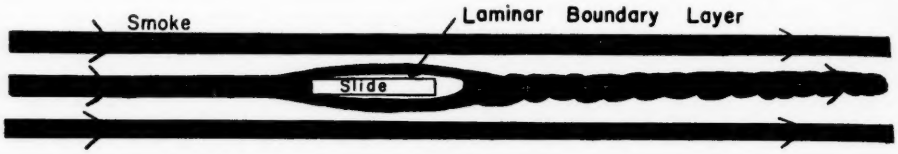


Fig. 2.—Schematic diagram of the flow of smoke around a microscope slide under laminar (nonturbulent) flow.

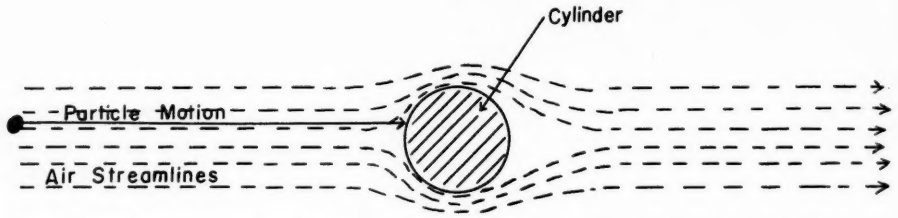


Fig. 3.—Schematic diagram of particle and air trajectories near a cylinder.

The size and shape of the slide holder will affect the flow of air around the sampler. In general, a bulky holder will produce a greater deflection of the airstream, resulting in a poorer catch.

The objections which have been raised to the use of the "gravity slide" in wind do not apply when the slide is placed in still air. Here neither orientation of the slide nor its shape can in any way alter the catch.

In the open air the height above ground at which the air begins to move varies from a fraction of a millimeter over a smooth flat surface to several meters in a dense forest. If a gravity slide is placed inside the layer of still air, the collection on the slide should represent the rate of deposition on a level surface. Unfortunately, the deposition may vary markedly from place to place due to minor undulations in the ground or to filtering action of the foliage.

THE NEW TECHNIQUES

History.—Rempe[7] and later Gregory[6] have shown that vertically oriented cylinders of an appropriate size were effective volumetric samplers under normal outdoor conditions. These samplers utilize the impingement principle. Particles moving in the air stream are carried across streamlines by their

inertia to strike the cylinder instead of following the flow around it (Fig. 3). A suitable adhesive placed on the cylinder retains the particles collected. Gregory demonstrated that the cylinder impingement efficiency* increases with increasing particle inertia and wind speed and with decreasing cylinder diameter.

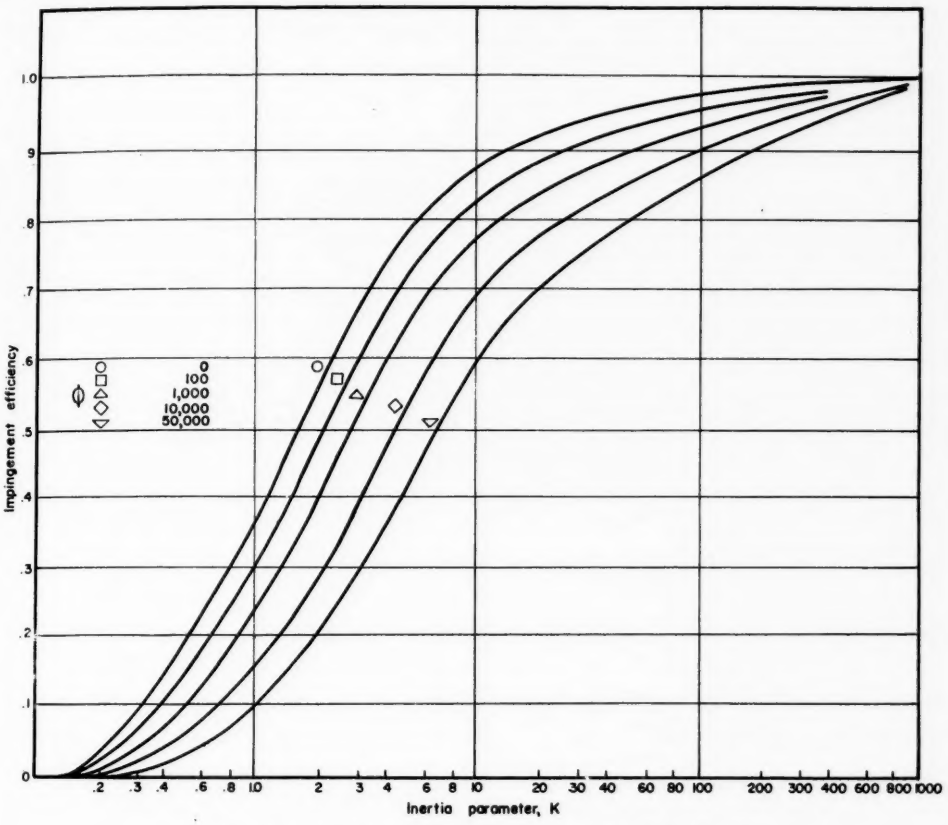


Fig. 4.—Cylinder impingement efficiency (NACA TN 2904).

Brun and Mergler[2] used an analogue computer to solve the linearized equations of motion for spherical particles impinging on a cylindrical collector. Fig. 4 shows the curve of impingement efficiency versus several properties of the particle, air, and sampler which have been combined into two dimensionless parameters, K and ϕ.

$$K = \frac{2}{9} \frac{\rho_p r^2 U}{\mu L}, \qquad \phi = \frac{18 \rho_a LU}{\mu \rho_p},$$

where ρ_p is the particle density, ρ_a the air density, r the particle radius, L the sampler radius, U the free stream velocity, and μ the air viscosity. (The

*Impingement efficiency = $\frac{\text{number of particles intercepted by cylinder}}{\text{number of particles initially in the volume swept out by the cylinder.}}$

equations and curve are included for those readers who may wish to compute collection efficiencies for particles or collectors different from those illustrated in the text.)

Fig. 5 shows the impingement efficiency of a cylinder of 1 mm. diameter for particles of unit density and various radii as a function of wind speed. It will be noted that a wire 1 mm. in diameter is a very satisfactory sampler for most of the common aeroallergens when the wind speed exceeds 2 m.p.h. For smaller particles or slower winds, a wire of smaller diameter must be used to maintain useful efficiencies.

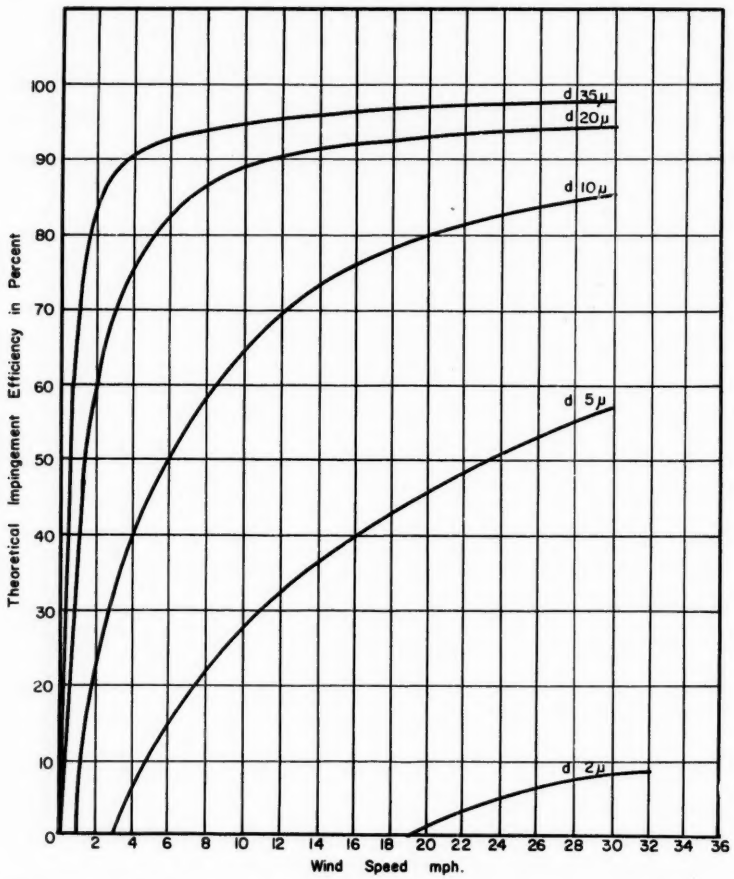


Fig. 5.—Impingement efficiency of a 1 mm. (diameter) cylinder for particles of unit density and various diameters.

The cylindrical samplers used by Gregory were satisfactory in the wind tunnel but impractical for use in the field. The curved surface of a cylinder is difficult to observe under a microscope, and if a removable coating is placed on the cylinder at least the side along which the coating opens is unusable. These difficulties have been largely overcome with the invention of the *flag sampler.*

The Flag Sampler.—Sakagami[8] has described a fast-response wind vane for use in micrometeorological studies. It consisted of a tiny paper vane turning about a vertical axis made of an ordinary household pin. The flag would follow up to sixty fluctuations in wind direction per second. By replacing the paper flag with double-coated cellulose (Scotch) tape, the Sakagami wind vane was converted into a flag sampler (Fig. 6). The flag keeps the same edge constantly facing into the wind. Particles impinge on this edge and are collected with the efficiency of a cylinder whose diameter is equal to that of the pin.

The glass bearing is simply a ¾ inch length of ⅛ inch glass tubing, sealed at its lower end and fire-polished at the top in order to create a low friction bearing. A variety of pins may be used, depending on the size of the particle being sampled. For particles larger than 15 μ a 2 inch pin one millimeter in diameter is satisfactory. A 2 inch length of double-coated, 1 inch cellulose tape is folded over the pin and a thin piece of doubled paper ¼ inch wide is inserted near the tail to facilitate separation of the tape after exposure. The leading edge of the sampler is coated with a dilute* solution of rubber cement. This procedure was adopted because wind-tunnel tests showed that rubber cement as a collecting surface is water proof and has an adhesive efficiency three to eight times that of the original cellulose tape surface.

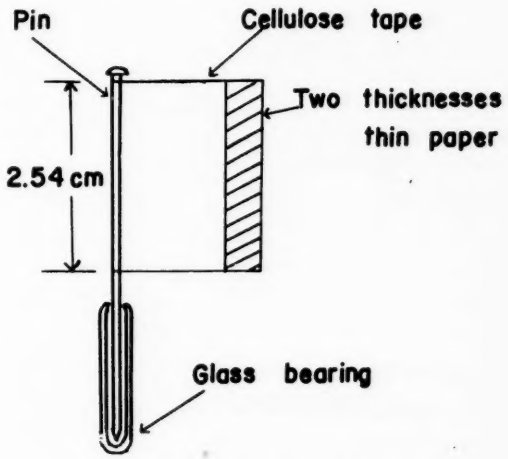


Fig. 6.—The flag sampler.

The flag sampler, mounted in its bearing, may be exposed in the atmosphere wherever sampling is desired. After exposure the flag may be stored to avoid future contamination of the sampling surface, or it may be mounted immediately on a standard microscope slide protected by a cover slip over the sample. If staining is desired, a thin strip of Calberla's solution† can be painted across the center of the cover slip before it is set in place.

*One part rubber cement to three parts rubber cement thinner.

†Calberla's solution: 1 part glycerine, 2 parts alcohol, 3 parts water, and 1 per cent basic fuchsin.

Under laminar flow in a wind tunnel, a 1 mm. pin collects all particles in a strip approximately ½ mm. wide. In turbulent flow outdoors, the sampler lags slightly in following the rapid fluctuations in wind direction. The catch is consequently spread over a band approximately 1½ mm. in width. This width corresponds to the field of view under an ordinary microscope using 100-power magnification. It is thus possible to count all particles collected on the flag sampler in a single longitudinal traverse of the sampled area by the microscope.

To compute the true concentration of a particulate from the flag count, it is necessary first to correct the count to the value that would have resulted if the sampler had been perfectly efficient. Then the corrected count divided by the volume sampled yields the true concentration:

$$\chi_T = \frac{C}{EV}, \text{ where } \begin{array}{l} \chi_T \text{ is the true concentration,} \\ C \text{ is the actual particulate count,} \\ E \text{ is the efficiency of the sampler,* and} \\ V \text{ is the volume sampled.} \end{array}$$

The volume sampled is given by:

$$V = A\bar{u}t, \text{ where } \begin{array}{l} A \text{ is the cross-sectional area of the sampler,} \\ \bar{u} \text{ is the mean wind speed, and} \\ t \text{ is the sampling period.} \end{array}$$

For example, a flag sampler 0.1 cm. in diameter and 2.54 cm. in height with a wind speed of 220 cm. per second (5 m.p.h.) will sample

$$\frac{0.1 \times 2.54 \times 220 \times 3600}{10^6} = 0.2 \text{ cubic meters per hour.}$$

If the particle being sampled has a density of 1 gram per cubic centimeter and a diameter of 20 μ, the impingement efficiency, at 5 m.p.h., will be 80 per cent (Fig. 5). Suppose that 100 particles are collected during the hour; the concentration:

$$\chi = \frac{100}{0.2 \times 0.8} = 625 \text{ particles per cubic meter.}$$

The advantages of the flag sampler are as follows:

1. It is sufficiently inexpensive to be useful in mass sampling programs.
2. Its impingement efficiency is known and is satisfactory over a range of particle sizes which include the common aeroallergens.
3. The small sampling area minimizes the chance of accidental contamination after exposure.
4. Counting time is reduced significantly.

The disadvantages include the following:

1. The flag sampler is inefficient at very low wind speeds, approaching zero efficiency as the wind becomes calm (Fig. 5).

*$E = E_i E_a$, where E_i is the impingement efficiency and E_a the adhesive efficiency. In the following example E_a will be taken as 1.00, although a figure near 0.40 is indicated by the wind-tunnel experiments.

2. The sampler is inefficient for very small particles (Fig. 5). (It is possible to sample for smaller particles by reducing the pin diameter, but there is a practical limit to this reduction at about ½ mm. because of the thickness of Scotch tape and frailty of the pin.)
3. The average wind speed must be known before the concentration can be calculated, and while the flag sampler itself may cost only a few cents, a recording anemometer is considerably more expensive.

The Roto-Bar Sampler.—The roto-bar sampler used at the University of Michigan is a modification of the excellent roto-rod sampler originally designed by personnel of the Stanford University Aerosol Laboratory.[1]

The principle of operation is much the same as for the flag sampler. A bar is rotated through the air at a constant speed. Particles impinge on the leading edge of the bar due to their inertia and are collected by a suitable adhesive (for example, dilute rubber cement).

The constant speed of rotation eliminates many of the disadvantages inherent in the flag sampler. Within reasonable limits, the collection efficiency of the roto-bar sampler is independent of wind speed.

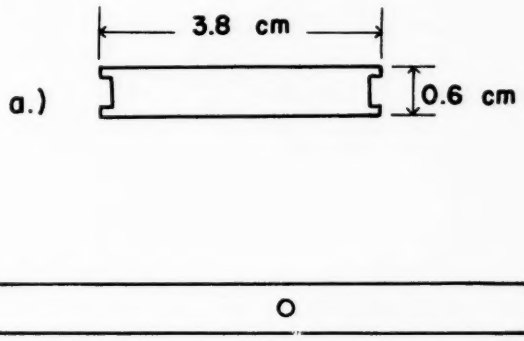


Fig. 7.—Two components of the roto-bar sampler. *A*, The sampling bar. *B*, Phosphor bronze spring holder for the sampling bar.

The sampler is a flat bronze bar (3.8 × 0.6 × 0.08 cm.) notched on either end (Fig. 7, *A*). The length and depth were chosen so that double-coated Scotch tape 1 inch wide could be applied to the bar and easily removed. The thickness was chosen so that the theoretical impingement efficiency would exceed 80 per cent for particles 10 μ in diameter and larger (Fig. 9).

The sampler bar is held by a pair of Phosphor bronze springs suitably notched and counterbalanced (Fig. 7, *B*). The springs are mounted on a shaft extension of a 3600 r.p.m., 110 volt AC, synchronous motor (Fig. 8).

A "coolie-hat" (conical) rain shield has been designed so that the sampler can be used during adverse weather. Only the smallest raindrops reach the sampler, and these do not appear to interfere with the collection efficiency.

As it is currently designed, the roto-bar unit samples nearly 1 cubic meter per hour. Conversion from count to concentration can be readily performed

by using the efficiency curve of Fig. 9 which was derived from Brun and Mergler's[2] data.

The relationship between roto-bar efficiency and particle diameter, as shown in Fig. 9, must be used with reservation. The curve was drawn according to Brun and Mergler's data for spherical particulates, a right circular cylindrical collector, and a perfect adhesive surface. In most cases none of these conditions is completely satisfied.

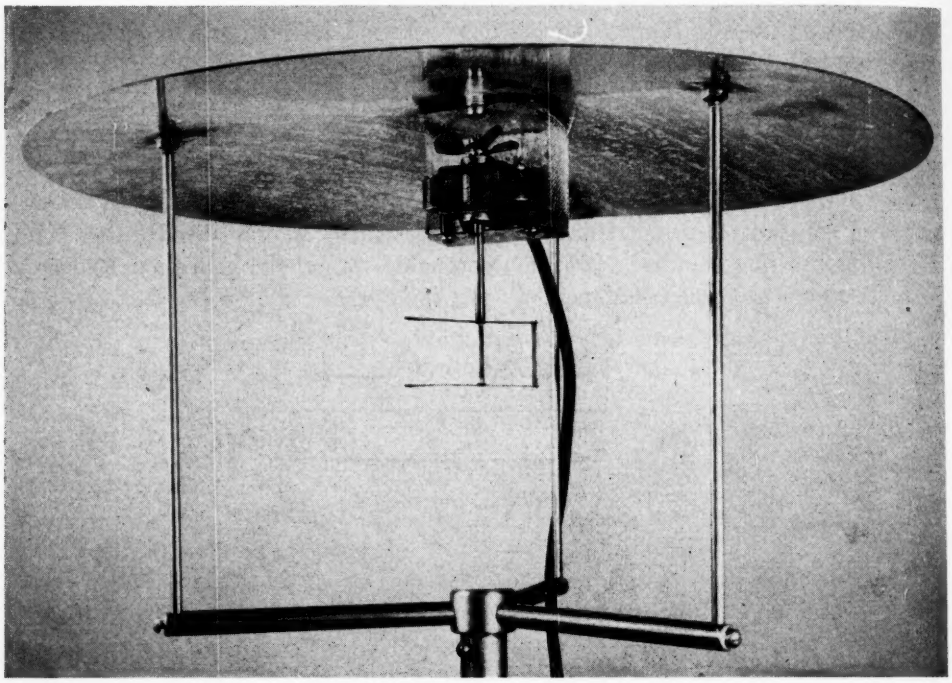


Fig. 8.—A complete roto-bar sampling unit with rain shield.

The sampling bar as currently designed is not cylindrical, but neither is it rectangular. During production deburring slightly rounds the edges of the bar, and when the cellulose tape is applied it springs slightly away from the leading edge of the bar. The net effect is to produce a curved impingement surface which may be considered to be of circular section.

Few of the common aeroallergens are spherical; ambrosia pollen probably is the closest and helminthosporium is the farthest from that ideal. If great sampling accuracy is desired, an equivalent spherical size could be computed for the irregular particulates by utilizing their known densities and fall speeds.

At present there is no perfect collecting surface for particulates as large as the common aeroallergens. Of the substances tested, dilute rubber cement seems the most promising. Wind-tunnel experiments are continuing both to measure the adhesive efficiency of rubber cement as a function of wind speed and to investigate other adhesive coatings.

The large volume sampled by the roto-bar can prove disadvantageous when a long sampling period is desired. For example, a roto-bar which is run continuously for twelve hours or more in a smoky or dusty atmosphere will collect so many particles that counting and recognition of the various granules becomes difficult.

Wind-Tunnel Tests.—Efficiency tests of several aeroallergen samplers were conducted in a wind tunnel of the closed-duct or return-flow type. Although the tunnel was originally designed for tests with aircraft models, wind speeds sufficiently low for meteorological applications can be generated. The test section is 4.5 meters long, 1.8 meters high, and 2.5 meters wide.

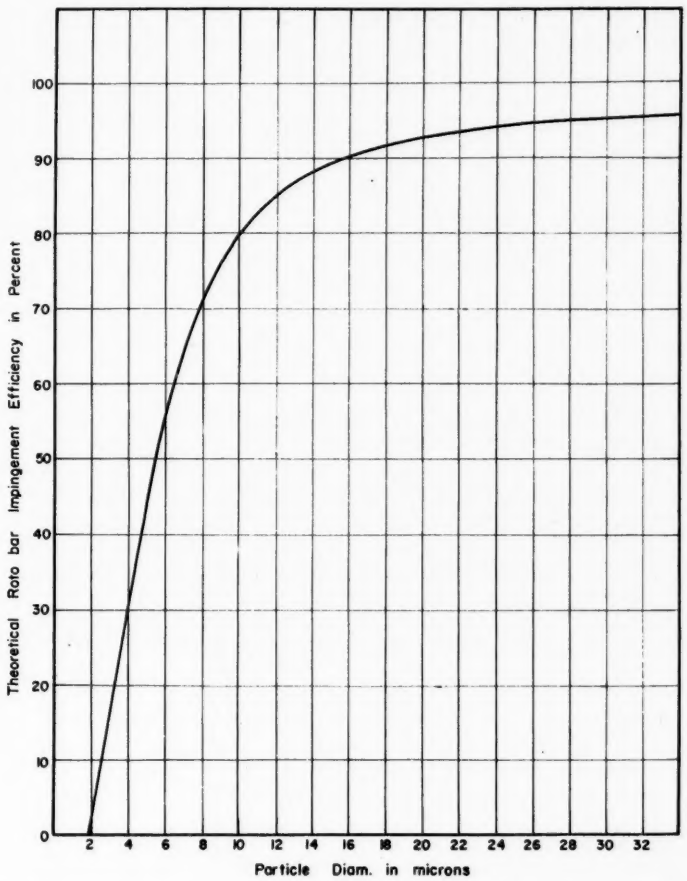


Fig. 9.—Roto-bar impingement efficiency for particles of unit density as a function of particle diameter.

Due to its closed-duct construction, the tunnel is ideal for efficiency testing of the type described below. The pollen is placed in the tunnel through a small hole in the ceiling just downwind from the samplers. The pollen then passes through the fan, through the return ducts, through the turbulence-reducing

meshes, and thence to the samplers. Concentrations were measured at 40 cm. intervals horizontally and vertically across the test section and found exceedingly uniform except in the 60 cm. nearest the tunnel walls.

The procedure followed in each experiment was as follows: Air in the tunnel was brought to the desired speed, and pollen samplers were placed in the test section. Pollen was then injected into the air stream, and roto-bars

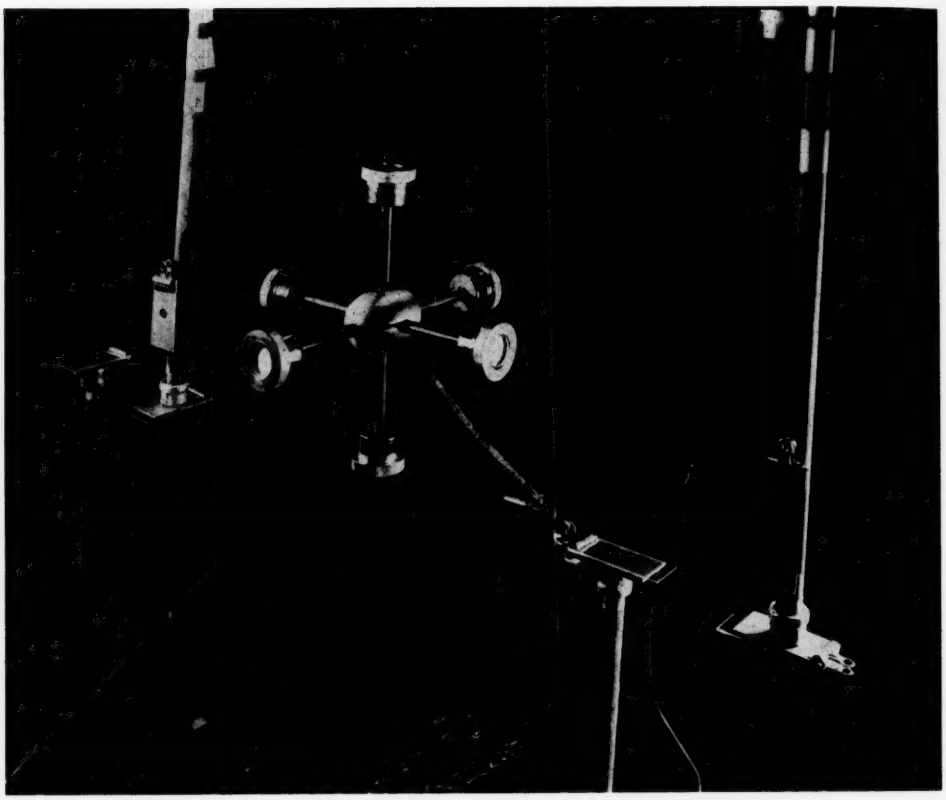


Fig. 10.—Experimental wind tunnel set-up showing the six-directional millipore filter unit with gravity slide samplers on either side.

and pumps for filters and impingers were started. After ten minutes the motors were stopped, and the samples were protected from further pollution as quickly as possible. The samples were then removed, and the wind speed was adjusted to a new value. The procedure was then repeated.

The possibility of error due to slight differences in exposure time of the various types of samplers was checked in the following way: A six-head millipore filter unit was placed, with the heads facing downward, in the test section and the timer was adjusted for a three-minute cycle. Wind speeds were reduced as low as possible (to 3 m.p.h.), so that the catch would be reasonably large. One head was run prior to placing pollen in the tunnel, and the remaining five were run in sequence for three minutes each. It was found that the

concentration was reduced to 0.7 per cent of its initial value in ten minutes and to 0.07 per cent in fifteen minutes. After fifteen minutes the concentration remained almost constant, probably due to reflotation from structural supports in the return duct and from the propellor. Thus, during removal of the samplers after exposure short differences in sampling time had a negligible effect upon the pollen catches.

A six-directional millipore filter unit was designed to measure the effect of orientation on collection efficiency (Fig. 10). The unit consisted of six millipore filter heads mounted so that the apertures faced into the wind, downwind, upward, downward, and to either side from an egg-shaped aluminum center piece 7.0 cm. long by 5.0 cm. in diameter. The unit was mounted in the center of the test section of the wind tunnel 45 cm. above the floor.

To ensure that the flow was equal through each head, heads with millipore filter in place were tested separately, and only those whose volumes were within 5 per cent of each other were chosen for each test. The heads were then covered with masking tape and screwed into place in readiness for the next test.

TABLE I. RESULTS OF A TYPICAL WIND-TUNNEL TEST DEMONSTRATING THE EFFECT OF ORIENTATION ON THE EFFICIENCIES OF GRAVITY SLIDE AND MILLIPORE FILTER SAMPLERS IN NONTURBULENT FLOW

TEST 8

DURATION OF TEST: 10 MINUTES
WIND SPEED: 670 CM. PER SECOND (15 M.P.H.)
TOTAL VOLUME THROUGH SIX-DIRECTIONAL UNIT: 0.645 CUBIC METERS

FACING	ORIENTATION OF LONG AXIS	COUNT	COMPUTED CONCENTRATION IN GRAINS PER CUBIC METER × 10^{-2}
	Slides		
Downward	Normal to wind	1	2
Downward	Parallel to wind	22	37
Upward	Normal to wind	17	29
Upward	Parallel to wind	530	900
Into the wind	Vertical	3,034	12
	Millipore Filters		
Facing left wall of tunnel		1	0
Facing right wall of tunnel		0	0
Facing upward		4	0
Facing downward		4	0
Facing into the wind		52,080	6,131
Facing down wind		7,825	921

Six coated microscope slides, held on brass plates by small clips, were mounted on either side of the millipore filter unit. Four slides, two facing upward and two facing downward, were placed at the level of the downward-facing millipore filter. Of the two slides in each direction, one was mounted with its long axis parallel to the air flow, and the other was mounted with its long axis normal to the air flow. The other two samplers were about 6.5 cm. higher and facing the wind (Fig. 10).

The collecting surface on the slide was double-coated cellulose tape, 2.54 cm. wide, placed across the center of the slide. (It was not realized until later that cellulose tape has a relatively low adhesive efficiency as compared to dilute rubber cement.)

TABLE II. RESULTS OF THREE WIND-TUNNEL TESTS COMPARING EFFICIENCIES OF GRAVITY SLIDES, MILLIPORE FILTERS, AND CYLINDERS UNDER SLIGHTLY TURBULENT FLOW

FACING	ORIENTATION OF LONG AXIS	TEST 10 TIME: 10 MINUTES WIND: 223 CM. PER SECOND VOLUME: 1.55 CUBIC METERS		TEST 11 TIME: 10 MINUTES WIND: 447 CM. PER SECOND VOLUME: 1.42 CUBIC METERS		TEST 12 TIME: 10 MINUTES WIND: 670 CM. PER SECOND (15 M.P.H.) VOLUME: 1.55 CUBIC METERS	
		COUNT	COMPUTED CONCENTRATION IN GRAINS PER CUBIC METER × 10^{-2}	COUNT	COMPUTED CONCENTRATION IN GRAINS PER CUBIC METER × 10^{-2}	COUNT	COMPUTED CONCENTRATION IN GRAINS PER CUBIC METER × 10^{-2}
			Slides				
1. Downward	Normal to wind	0	0	28*	–	0	0
3. Downward	Parallel to wind	2	3	1	2	0	0
2. Upward	Normal to wind	8	14	3	5	1	2
4. Upward	Parallel to wind	744	1,265	186	316	45	77
5. Into the wind	Vertical	3,850	58	1,118	8	441	2
			Millipore Filters				
7. Facing left wall of tunnel		3,653	307	21	2	21	2
9. Facing right wall of tunnel		9,442	794	89	8	53	5
11. Facing upward		5,859	493	61	6	15	1
12. Facing downward		4,066	342	59	5	32	3
8. Facing into the wind		73,500	6,180	6,257	566	1,975	166
10. Facing down wind		23,100	1,942	945	85	236	20
			Cylinders				
Pipe 17.4 mm. diameter		5,470	92	4.871	42	485	3
Wire 1.0 mm. diameter		3,310	971	1,298	190	826	81

*High count appears to be due to accidental pollution.

Many tests were run at various wind speeds, both with and without baffles. The purpose of the baffles was to induce turbulence in the air stream similar to that encountered outdoors. Later tests were run outdoors; hence, the tunnel tests with baffles are not reported here. All tests showed much the same result, a typical one being illustrated in Table I.

The technique used to convert pollen count to concentration varied with each type of sampler. Horizontal gravity slides were assumed to sample a volume equal to the product of the fall speed of the pollen (1.6 cm. per second), the area of the sampling surface, and the time. Vertically oriented slides were assumed to sample a volume equal to the product of the area of the sampling surface, the wind speed, and the time. The millipore filters, regardless of orientation, were each assumed to sample one-sixth of the total volume of air drawn through the six-directional unit.

Three main conclusions can be drawn from the dramatic results displayed in Table I and reproduced in eleven other tests:

1. Horizontal microscope slides positioned in a nonturbulent air stream are poor samplers. They not only are inefficient, but the efficiency varies markedly with orientation.

2. Millipore filters oriented normal to a nonturbulent wind have extremely low efficiencies except when the wind is nearly calm. (For a curve showing millipore filter efficiency as a function of wind speed, see Fig. 11.)

3. Filters facing into the wind are highly efficient, catching up to 10,000 times the number of particles caught by filters facing normal to the wind. (Problems associated with this type of sampling have been mentioned earlier.)

The extraordinarily high counts on the forward-facing samplers led to experimentation with vertically oriented cylinders. Two sizes were used, pipe 17.4 mm. in diameter and wire 1.0 mm. in diameter. Both were wrapped with cellulose tape coated with dilute rubber cement. Dilute rubber cement was also applied to the cellulose tape on the microscope slides in these experiments. Typical results are shown in Table II.

No significance should be attached to differences in count between experiments, since the amount of pollen injected into the tunnel varied from one experiment to another.

During tests 10, 11, and 12, a grid of lath 3.8 cm. wide, 1.3 cm. thick, and spaced 20.3 cm. apart was placed across the entire throat of the test section in order to induce small-scale turbulence in the air stream. A Gill bivane[5] was used to compare turbulence generated in the tunnel with normal turbulence outdoors. The bivane recorded a very low-amplitude trace in the tunnel while outdoors, even with stable air and light winds, the amplitude was two orders of magnitude larger.

The slight turbulence present during these three tests, together with an increase in air velocity through the filters, resulted in an increased catch on

the normal-facing filters as compared to the forward-facing filter. Otherwise, the results for gravity slides and filters closely resemble those for test 8 shown in Table I.

Concentrations computed from the catch on the 1 mm. wire sampler were of the same order of magnitude as those computed from the catch on forward-facing filters.

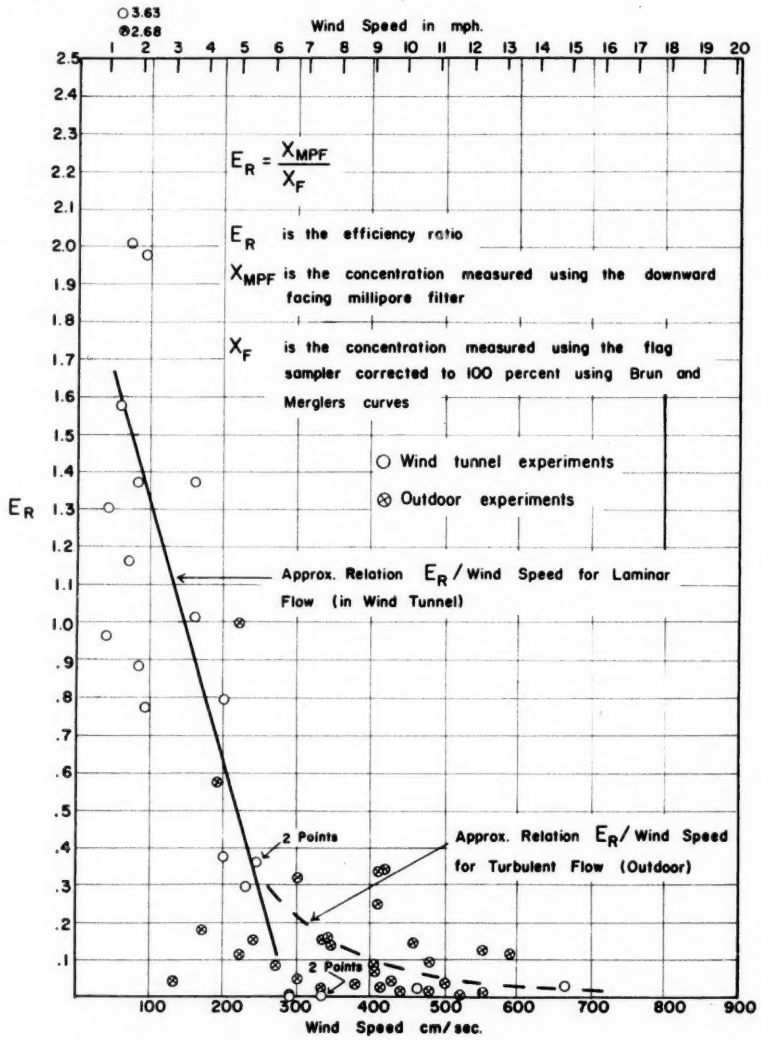

○3.63
⊗2.68
Wind Speed in mph.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 .9 .8 .7 .6 .5 .4 .3 .2 .1
E_R
$E_R = \frac{X_{MPF}}{X_F}$
E_R is the efficiency ratio
X_{MPF} is the concentration measured using the downward facing millipore filter
X_F is the concentration measured using the flag sampler corrected to 100 percent using Brun and Merglers curves
○ Wind tunnel experiments
⊗ Outdoor experiments
Approx. Relation E_R / Wind Speed for Laminar Flow (in Wind Tunnel)
2 Points
Approx. Relation E_R / Wind Speed for Turbulent Flow (Outdoor)
2 Points
100 200 300 400 500 600 700 800 900
Wind Speed cm/sec.

Fig. 11.—Experimental millipore filter impingement efficiency as a function of wind speed. Efficiencies greater than 100 per cent are the result of imperfect adhesiveness of the dilute rubber cement surface on the flag samplers.

Further experiments were conducted to determine the reliability of the wire sampler. A typical test in which five samplers were exposed at the same height across the center of the wind-tunnel test section is illustrated in Table III.

Outdoor Tests.—Outdoor experiments were conducted to measure the effect of natural turbulence on sampler collection efficiency. Two series of tests were run, the first using commercial pollen dispensed from a tube by compressed air and the second conducted during the regular ragweed season.

TABLE III. THE CATCH ON FIVE 1 MM. DIAMETER WIRE SAMPLERS PLACED AT THE SAME ELEVATION ACROSS THE WIND TUNNEL TEST SECTION

Counts	1,809	1,862	2,007	1,992	1,822
Mean	1,899	Standard deviation: 70 = 4%			

During the first series of experiments, the samplers were mounted 0.6 meter above ground level in an array 3 meters long by ½ meter wide. Pollen was dispensed 30 meters upwind from the array so that the cloud of pollen passing the array would be uniformly dispersed. A plume of smoke, made by mixing ammonia and sulfur dioxide gases, made it possible to follow the pollen plume visually.

Meteorological instrumentation included the following:

1. Thermocouples at ½, 1, 2, and 4 meters.
2. Anemometers at ½, 1, 2 and 4 meters.
3. A bivane at 1 meter.

Accurate measurement of lapse rate, wind speed, and turbulence was possible.

The second series of experiments was conducted during the regular ragweed season. A flag, a roto-bar, and a downward-facing millipore filter sampler were mounted 3.7 meters above a weedy field. An anemometer at the same level

TABLE IV. RESULTS OF TWO TESTS CONDUCTED OUTDOORS OVER LEVEL GROUND WITH COMMERCIAL RAGWEED POLLEN

	TIME: 10 MINUTES WIND: 58 CM. PER SECOND (1.3 M.P.H.) FILTER VOLUME: 0.26 CUBIC METER		TIME: 10 MINUTES WIND: 460 CM. PER SECOND (10.3 M.P.H.) FILTER VOLUME: 0.05 CUBIC METER	
SAMPLER	COUNT	COMPUTED CONCENTRATION IN GRAINS PER CUBIC METER × 10^{-2}	COUNT	COMPUTED CONCENTRATION IN GRAINS PER CUBIC METER × 10^{-2}
Glass slide, long axis normal to the wind	760	1,238	15	26
Glass slide, long axis parallel to the wind	1,129	1,852	67	109
Downward-facing millipore filter	9,793	376	11	2
Flag sampler	582	66	97	14

continuously recorded the wind speed. The three samplers were operated simultaneously for a two-hour period from 10 o'clock to 12 o'clock E.S.T. on thirteen days, beginning Sept. 1, 1958.

The results of the tests corroborate the wind-tunnel findings mentioned earlier. Millipore filter efficiencies were higher than indicated in wind-tunnel studies (Fig. 11), but they were still much below those of the flag and roto-bar

samplers, except at very low wind speeds. The increased efficiency of the millipore filter outdoors is apparently due to the effect of impaction of pollen on the samplers by turbulent eddies. The dependence of millipore filter catch on wind speeds is clearly indicated by the results of many experiments, eight of which are illustrated in Tables IV and V.

TABLE V. RESULTS OF SIX TESTS CONDUCTED OUTDOORS 3.7 METERS ABOVE A WEEDY FIELD DURING THE NORMAL RAGWEED SEASON

SAMPLER	WIND SPEED	COUNT	χ IN GRAINS* PER CUBIC METER	WIND SPEED	COUNT	χ IN GRAINS PER CUBIC METER
Millipore filter	3.0 m.p.h.	8	18	9.9 m.p.h.	12	28
Flag	134 cm./sec.	46	187	443 cm./sec.	964	1,190
Roto-bar		170	92		790	424
Millipore filter	5.4 m.p.h.	68	148	10.8 m.p.h.	63	134
Flag	241 cm./sec.	288	653	483 cm./sec.	987	1,119
Roto-bar		1,232	660		2,529	1,353
Millipore filter	8.5 m.p.h.	7	14	12.1 m.p.h.	7	14
Flag	380 cm./sec.	195	279	541 cm./sec.	2,209	2,232
Roto-bar		272	145		3,266	1,748
Millipore filter	9.1 m.p.h.	128	286	12.4 m.p.h.	3	7
Flag	407 cm./sec.	2,573	3,289	554 cm./sec.	434	427
Roto-bar		5,824	3,115		399	212
Millipore filter	9.3 m.p.h.	172	371	13.2 m.p.h.	40	88
Flag	416 cm./sec.	650	855	590 cm./sec.	697	646
Roto-bar		1,319	706		930	498
Millipore filter	9.3 m.p.h.	5	11	18.1 m.p.h.	6	14
Flag	416 cm./sec.	214	279	809 cm./sec.	2,256	1,526
Roto-bar		435	233		2,773	1,483

Duration of each test: 120 minutes.
Filter volume for each test was 0.45 ± 0.01 cubic meter.
*No correction was made for adhesive or impingement efficiency.

A comparison of roto-bar and flag sampler results showed a surprising agreement in the calculated concentrations (Table V). Since no correction for impingement efficiency has been made, it was expected that the roto-bar with its high speed of rotation (and resulting high impingement efficiency) would indicate higher concentrations than the flag sampler, particularly at low wind speeds. This expectation was not borne out by the observations. The diminution of the roto-bar catch is likely the result of low adhesive efficiency at the high peripheral speed of the roto-bar sampler. Nevertheless, both flag and roto-bar samplers are seen to give uniform and reliable measurements of pollen concentration.

CONCLUSIONS

1. The gravity slide is not a suitable instrument for measuring concentrations of particulates in the air. If gravity slides must be used, they should be mounted level with the ground where wind speeds are virtually zero.

2. Samplers which draw air through an aperture are not recommended for outdoor sampling unless the particle being sampled is extremely small (at least under 5 μ).

3. The flag sampler is an excellent instrument for use in large-scale sampling programs or at other times when a roto-bar sampler is not available. Its limitations in very light winds or for very small particles must be considered in calculating true air concentrations.

4. The roto-bar sampler has very distinct advantages over previous outdoor sampling techniques and is highly recommended for pollen- or spore-sampling programs. Its efficiency is semi-independent of wind speed, so that roto-bar counts can be used directly to calculate concentrations without recourse to an anemometer record.

The authors wish to thank Dr. E. W. Hewson, Director of the Meteorological Laboratories, for his encouragement and many helpful suggestions. Appreciation is expressed to Mr. K. R. Hardy for his assistance in several field tests, to Mr. D. L. Jones for his helpful suggestions in composing the paper, and to Mrs. Katalin Racz who abstracted the meteorological data, supervised the pollen counting, and drafted the figures.

REFERENCES

1. Aerosol Laboratory. Stanford University: The Roto-rod Sampler, Second Semiannual Report, CML 186, Stanford, Calif., 1957, Stanford University Press.
2. Brun, R. J., and Mergler, H. W.: Impingement of Water Droplets on a Cylinder in an Incompressible Flow Field and Evaluation of Rotating Multicylinder Method for Measurement of Droplet-Size Distribution, Volume-Median Droplet Size, and Liquid-Water Content in Clouds, Technical Note 2904, National Advisory Committee for Aeronautics, Washington, D. C., 1953.
3. Crawford, J. H.: Determination of the Specific Gravity of Ragweed Pollen (Ambrosia Elation) and Conversion of Gravity of Sample Counts to Volumetric Incidence, Pub. Health Rep. **64**: 1195-1200, 1949.
4. Durham, O. C.: The Volumetric Incidence of Atmospheric Allergens, IV. A Proposed Standard Method of Gravity Sampling, Counting and Volumetric Interpolation of Results, J. Allergy **17**: 79-86, 1946.
5. Gill, G. C., and Cole A. L.: A Precision Bivane for Air Pollution Studies, Paper Presented at Second National Conference on Applied Meteorology: Engineering, 1958.
6. Gregory, P. H.: Deposition of Air-Borne Lycopodium Spores on Cylinders, Ann. Applied Biol. **38**: 357-376, 1951.
7. Rempe, H.: Untersuchungen uber die Verbreitung des Blutenstaubes durch die Luftstromungen, Planta **27**: 93-147, 1937.
8. Sakagami, J.: On the Structure of Atmospheric Turbulence Near the Ground, Nat. Sci. Reports, 1951, Ochanomizu University, Tokyo, vol. 1, pp. 40-50.
9. Sutton, O. G.: Micrometeorology, New York, 1953, McGraw-Hill Book Company, p. 254.

EDITORIALS

Primum non Nocere

We owe much to the big drug houses; we could not practice modern medicine without them. They are our friends and, in a sense, our partners. Furthermore, they are sincerely devoted to developing the best new products possible and are continually striving to improve those they already have. Their achievements have been truly colossal.

It must be remembered, however, that in order to make money for their stockholders, which is certainly one very important reason for their existence, it is necessary that they have a steady stream of *new* products, just as a publishing house continually has to have new books. It is also necessary that these new products sell in quantity, even if only for a few months. This has to be brought about in the present day and age by up-to-date high-power advertising to doctors by means approaching those used by Madison Avenue to sell automobiles, soap, breakfast foods, or whiskey to the laity. Due to almost incredible advances in organic chemistry, the synthesis of an amazing array of drugs has been accomplished—cortisone and its numerous siblings, the antibiotic family, tranquilizers, vitamins, cough stoppers, old-age preventers, blood pressure reducers, antiwheezers, antisneezers, antiseptics, and dozens more, many with five or six different trade names of weird etymology. By the introduction of a benzol ring here, the addition of a CH_3 group there, or a rearrangement of OH groups somewhere else, it has become possible to keep turning out innumerable new compounds which work on the human organism in almost any way desired. The trouble is that they too often work in ways which are not desired. Hence, penicillin anaphylaxis and urticaria, chloramphenicol neutropenia, phenothiazine convulsions and neutropenia, steroid duodenal ulcer, and so on.

Although one must admit that the only drug which cannot produce an adverse reaction in some people is a drug which has no therapeutic action either, there are altogether too many adverse reactions from some of the newer synthetic drugs; the patient is too often made sicker by the medicine than he was by the disease. The tendency in much of the advertising of new drugs is to overplay the desired effects and to underplay the undesired effects; this is natural enough.

This is the era we are in and will continue to be in. We have changed from a time of therapeutic nihilism to a time of therapeutic frenzy. It behooves us to be alert to progress and to welcome genuine advancement but

to be equally alert not to do our patient more harm than good when we prescribe for him some half-baked synthetic drug just because it is new, when it is not yet "dry behind the ears" and has not been in existence long enough to have exhibited its true virtues and defects.

L. W. H.

Abstracts for the Annual Meeting of the American Academy of Allergy, Jan. 11, 12, and 13, 1960

THE next annual meeting of the American Academy of Allergy will take place one month earlier than has been customary in previous years. This will force the Program Committee to make its selection of papers for the scientific program earlier than has been the case heretofore. In past years, the Program Committee has allowed some flexibility regarding the time of receiving abstracts, but this year the Committee will not be in a position to depart from the announced policy of considering for inclusion in the program only those abstracts received on or before the deadline.

The interest and value of the annual meeting is directly dependent on the papers presented in the scientific session. It is therefore most important that the change in the date and the need to adhere rigidly to the deadline be kept in mind so that abstracts will be submitted in time for consideration.

Abstracts should be not more than 250 words in length, should be submitted in triplicate, and should reach the American Academy of Allergy, Executive Office, 756 N. Milwaukee St., Milwaukee 2, Wisconsin, not later than Monday, Aug. 31, 1959.

OBITUARY

Selian Hebald

SELIAN HEBALD, a Fellow of the Academy of Allergy since its formation, died on Feb. 17, 1959, at the age of 63 years. Dr. Hebald graduated from Columbia University College of Physicians and Surgeons in 1919 and served as a resident physician at Mt. Sinai Hospital in New York City. Soon thereafter he became interested in allergy, and in 1923 he joined the staff of Dr. Robert A. Cooke's allergy clinic, then at the New York Hospital. In 1924 he was elected a Fellow of the Association for the Study of Asthma and Allied Conditions. He remained associated with Dr. Cooke's clinic when it moved to Roosevelt Hospital, where he served as assistant physician. He also conducted an allergy clinic at the Hospital for Joint Diseases, where he was Chief Emeritus of Allergy at the time of his death.

Dr. Hebald was a loyal and active member of the Academy, recently serving as chairman of the Credentials Committee. He was also a Fellow of the New York Academy of Medicine and a member of the New York Allergy Society and other medical organizations.

His primary interest was always in the practice of allergy, but he also participated in the research work at Roosevelt Hospital, including the early work on blocking antibodies. He contributed chapters on hay fever to two allergy textbooks: *Allergy in Theory and Practice* (W. B. Saunders Company, Philadelphia) and *Fundamentals of Modern Allergy* (McGraw-Hill Book Company, New York).

Dr. Hebald was well liked and respected by his associates and will long be remembered for his pleasant manner and cheerful outlook. The Academy will miss him.

He is survived by his wife, Mrs. Edith Lipsig Hebald; a daughter, Miss Anne Hebald; a son, Selian Hebald, Jr.; and a sister, Mrs. Jacob Birnbaum.

W. B. Sherman, M.D.

BOOK REVIEW

Occupational Allergy. Lectures Held During a Course on Occupational Allergy at the Hague in May, 1958, Organized by The Netherlands Society of Allergy in cooperation with the Netherlands Institute for Preventive Medicine and the Netherlands Society of Occupational Medicine under the Auspices of the European Academy of Allergy. Published by Charles C Thomas, Springfield, Ill., 1958. Copyright 1958 by H. E. Stenfert Kroese N. V., Leiden, Holland.

This book is a must for all those interested in occupational diseases, their prevention, and their treatment. It will surely interest the allergist, the dermatologist, and the specialists in pulmonary diseases, nasal diseases, industrial hygiene and engineering, and indeed many others.

As W. Jadassohn states in the introduction: "I do not believe that there is a single clinical specialty where one is not called upon to resolve allergy problems or at least to consider them." D. A. Williams, in his chapter, quotes Bernardine Ramazzini, the seventeenth century father of industrial medicine in the following statement:

> Medicine, like jurisprudence, should make a contribution to the well-being of workers and see to it that, so far as possible, they should exercise their callings without harm. So I for my part have done what I could and have not thought it unbecoming to make my way into the lowliest of workshops and study the mysteries of the mechanic arts. When a doctor visits a working-class home, he should be content to sit on a three-legged stool if there isn't a gilded chair, and he should take time for his examination; and, to the questions recommended by Hippocrates he should add one more: "What is your occupation?"

These statements, far from being weakened by the advances of civilization, must be strengthened and intensified in the mind of every physician in view of the tremendous number of new allergens which are constantly being introduced, not only in various occupations but also into everyday life—the home, food, clothing, detergents, cosmetics, and myriads of other diverse and incessant exposures of modern man.

This book, in unique fashion, covers and discusses a multitude of the problems in this highly variegated field. Of the thirty-seven different sections contributed by experts

from many different countries of Europe, thirteen are devoted to general problems of occupational allergy, twelve to occupational respiratory allergy, eleven to cutaneous allergic manifestations related to occupation, and one to allergic occupational manifestations of the eye. The following are a few examples of the many topics discussed: definitions; the impact of allergic occupational diseases upon society; the need for skin testing; the techniques and pitfalls of patch tests; occupational diseases, both according to the various occupations and according to the causal allergens; pre-employment examinations; the examination of patients for allergy; problems of compensation; the role of the skin as a protective organ; and the buffer action of the skin. There are also many chapters that teach a great deal about the fundamental aspects of the diseases concerned. An example is the very fine, though brief, discussion of the occupational problems of patients with Besnier's prurigo (atopic dermatitis). In the field of prevention and compensation for allergic diseases, the sixteen points of view on the prevention and compensation of allergic occupational diseases are in themselves worthy of being engraved in the minds of every industrial physician, if not framed and hung in every doctor's office and in every factory. In this chapter the statistics given for France of today are worthy of quotation. Each year there are in France about 10,000 cases of occupational disease as against one million cases of occupational accident (involving interruption of work for more than twenty-four hours). About 90 per cent of the 10,000 cases of occupational disease involve an allergic mechanism. Nearly 70 per cent of this group are dermatoses, 25 per cent are respiratory affections, and 5 per cent are other disorders. It will be seen that here, as always, occupational dermatoses are more than twice as common as all other occupational diseases combined.

The contributions of the different authors are of such uniformly high quality that it is an injustice to single out any of them by name. However, the medical profession will be personally most directly interested in the very excellent contributions on *occupational eczema in therapists* (including physicians) and the stimulating section on *allergic reactions in nurses*. The same remark applies to the sections on the various kinds of drug allergies in those who professionally handle and administer the drugs; the discussion of the dental allergies; and the section on respiratory allergy in the medical profession and its auxiliaries. As Gardiner is quoted by Herman Michel of Berlin as saying: "The investigation of allergic problems requires 'the inquisitiveness of a Sherlock Holmes, the genius of a Thomas Edison, the patience of Diogenes, and the optimism of an idiot.'" This reviewer believes there should be added to this list the fact that those who wish to unravel the complex problems of allergic occupational diseases must have an exhaustive understanding of *all* medicine, as well as of such basic sciences as immunology, microbiology, biochemistry, etc. In addition, they should know a great deal about industrial processes and almost everything that is to be known about the world which surrounds the worker, either at his home, at his occupation, or at his hobbies and recreations—in fact, through every waking and sleeping hour of his life. This being the case, one sees that allergy and industrial medicine are both, as W. Jadassohn had stated in his introduction, "cross section specialties (Querschnittsfächer)," not only of medicine but of the world. Indeed they cut across every aspect not only of medicine but of more than medicine—of the legal, industrial, manufacturing, household, and recreational complexities with which civilized man is beset.

While the present book does not aim at being a complete treatise on all of these, or indeed on any of these, each aspect is touched upon with sufficient imagination and sufficient detail to make this text a fine place to begin to delve into the mysteries of allergy as related to occupational exposures.

Marion B. Sulzberger, M.D.

ANNOUNCEMENTS

Committee on Food Allergy Seeks Evidence of Cottonseed Oil Reactions

The Committee on Food Allergy of the Research Council of the American Academy of Allergy is vitally interested in the problem of allergy to cottonseed oil and the current attitude of the Food and Drug Administration. In this regard, the Committee is very desirous of collecting documented evidence of clinical allergic reactions to cottonseed oil occurring in patients who may or may not have associated cottonseed protein sensitivity. The valued cooperation of any physician who may be able to furnish such case histories is urgently requested and desired.

Please direct such relative information to the Committee chairman, Dr. Sheldon G. Cohen, 309 South Franklin St., Wilkes-Barre, Pennsylvania.

Buffalo Allergy Society

The Buffalo Allergy Society announces that its new officers for 1959-1960 are as follows:

President—Victor L. Cohen, 341 Linwood Ave., Buffalo, New York.
Secretary—Leonard Wolin, 1201 Colvin Ave., Buffalo, New York.
Treasurer—Thelma Brock, 964 Delaware Ave., Buffalo, New York.

Michigan Allergy Society

The Michigan Allergy Society announces the following officers for the year 1959-1960:

President—Milton J. Steinhardt, Detroit, Michigan.
Vice-President—Robert G. Lovell, Ann Arbor, Michigan.
Secretary—Alex S. Friedlaender, Detroit, Michigan.
Treasurer—Hilda Hensel, Monroe, Michigan.

New Jersey Allergy Society

The New Jersey Allergy Society annonuces election of the following officers:

President—Frank L. Rosen, Newark, New Jersey.
Vice-President—Joseph R. Jehl, Clifton, New Jersey.
Secretary—Herbert W. Diefendorf, Summit, New Jersey.
Treasurer—Francis A. Pflum, Asbury Park, New Jersey.

West Virginia State Society of Allergy

The second annual meeting of the West Virginia State Society of Allergy will be held on Aug. 20, 1959, at the Greenbrier Hotel, White Sulphur Springs, West Virginia. The meeting will begin at 2 P.M.